THE PREGNANEDIOL EXCRETION IN NORMAL AND ABNORMAL PREGNANCY

THESIS

PRESENTED IN FULFILMENT OF

THE REQUIREMENTS FOR THE

DEGREE

OF

DOCTOR OF MEDICINE

OF

THE UNIVERSITY OF GLASGOW

BY

MARY G. COYLE, M.B.Ch.B., M.R.C.O.G.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

CONTENTS.

GENERAL INTRODUCTION.

For many years it had been clear that a reliable means of assessing placental function would provide clinicians with a valuable tool, in controlling treatment and guiding the prognosis, in abnormal pregnancy conditions. The placenta is known to produce an enormously wide variety of substances, directly or indirectly influencing foetal development. Some of these are incapable of assessment and are not produced continuously during pregnancy. Others are in constant production and because they give rise to well recognised excretion products lend themselves to a study of placental function. Oestrogens and progesterone are examples of such products, but whereas the chemical nature of the various end products of oestrogen metabolism has not been fully investigated, it has long been recognised that sodium pregnanediol glycuronide forms a major part of the katabolites derived from progesterone.

Various methods have been formulated for the urinary pregnanediol estimation. It may be estimated in conjugated form, Sodium Pregnanediol Glycuronide /Na.P.G./ (Venning, 1937, Allan &

Viergiver, 1941; Kaufman & Westphal, 1947) or as free pregnanediol (Davis & Fugo, 1947; Guterman 1947; Sommerville, Gough & Marrian, 1948; de Watteville, 1952, Klopper, Michie & Browne, 1955). Before this work was undertaken considerable controversy existed regarding the normal range of values and the clinical significances of pregnanediol excretion. Thus the values given for the peak excretion in the last months of pregnancy vary according to different workers:- Vemning (1937) 72 $\pm$ 22, Davis & Fugo (1947) 73 $\pm$ 12, Michie (1953) 62 $\pm$ 21, de Watteville (1951) 49$\pm$ 12 and Kaufman, Westphal & Zander (1951) 22 $\pm$ 22.

Pregnanediol is one of the excretory end products of progesterone metabolism produced in the secretory phase of the normal menstrual cycle and throughout pregnancy. Progesterone is essential for the embedding and growth of the early ovum. Many observers have thought that lack of progesterone was related to imperfect embedding and abortion, the pregnanediol assay has been advocated as a guide to prognosis and therapy in threatened and recurrent abortions (Cope, 1940; Hain, 1941 and Bender, 1948). Others have found it of limited value in these

conditions Swyer & Daly (1953) and de Watteville (1951).

The placenta takes over the function of the corpus luteum after the 3rd month of pregnancy where a rise in the level of pregnanediol excretion is noted. Late pregnancy disorders may be associated with a drop in progesterone production and hence a fall in the pregnanediol excretion. Smith & Smith (1954) showed that when regular assays were performed on 104 normal pregnant women the excretion dropped or failed to rise 3 to 5 days before the patients showed signs of toxaemia. Venning (1938); Hain (1941); and de Watteville (1951) failed to find any correlation between the pregnanediol excretion and the degree of toxaemia, but they found that in certain cases where there was danger of intrauterine foetal death there was also a low pregnanediol excretion. Trolle (1955) suggested that the presence of albumen in the urine in these cases might give rise to fallacious results, The albumen inducing, during toluene extraction, the formation of a firm emulsion, making recovery of pregnanediol difficult.

In chronic nephritis associated with essential hypertension, Pigeaud, Burthiault & Bertoux (1954) found that the pregnanediol excretion was low if intrauterine foetal death occurred. This was not so when chronic nephritis alone was present.

White & Hunt (1940), White (1949) and Smith & Smith (1947) showed that in the diabetic patient low pregnanediol values were associated with intrauterine foetal death. Peel (1955) stated that a fall in excretion of pregnanediol preceeded the foetal death in diabetes, but Gray (1955) was unable to show any significant difference between the results obtained in diabetic and in normal pregnant women.

The conflicting views in the literature cited showed that no clear picture of the normal range of pregnanediol excretion in pregnancy or of the variations which occur in early or late abnormal pregnancy could be obtained, and it was decided therefore to make a more comprehensive study of the problem. The first part of the work comprises an investigation into the variations which occur

when different methods are used to extract and purify pregnanediol. A review of the literature showed that by the method used by Sommerville et al (1948) a purer extract was obtained than by any of the shorter methods. This method was chosen for comparison with a modification of the method of Henderson et al (1949) which had been in current use at the Jessop Hospital for Women, Sheffield for the previous five years. Since neither of these methods proved to be satisfactory, an analysis was made of a new chromatographic method by Klopper, Michie & Brown (1955). This method was found to give a purer extract and it was adopted subsequently for all the work on the pregnanediol excretion in normal and abnormal pregnancy described below.

The investigation fell into 4 divisions:-

SECTION I. METHODS.

Part A. Methods of Determining the Urinary Pregnanediol. This consists of a comparison between the method of Sommerville, Marrian and Gough (1948), and of Mitchell (1954).

Part B. Critical review of the method of Klopper, Michie and Brown (1955).

Part C. The relationship between the Blood "progestational activity", estimated by the Hooker and Forbes (1947) biological assay and the urinary pregnanediol excretion in normal pregnancy.

SECTION II. NORMAL PREGNANCY.

The range of pregnanediol excretion during normal pregnancy, before the onset of labour and in the puerperium was investigated.

SECTION III. ABNORMAL EARLY PREGNANCY.

The pregnanediol excretion in cases of threatened abortion, recurrent abortion and in Hydatidiform mole was investigated.

SECTION IV. ABNORMAL LATE PREGNANCY.

Part A. The pregnanediol excretion in the Toxaemias of Pregnancy. The following conditions were investigated:-

A) Pre-eclamptic toxaemia

B) Essential Hypertension

C) Stillbirths and neonatal deaths associated with Toxaemia

D) Chronic renal disease

Part B. The pregnanediol excretion in conditions associated with foetal death in late pregnancy. The following conditions were investigated:-

A) Antepartum Haemorrhage

B) Diabetes mellitus

C) Rhesus immunisation including cases of unexplained foetal death

D) Bad obstetric history.

Each section begins with a short introduction which includes references to the literature and it is concluded by a general discussion. A summary of the work reported and the conclusion drawn from it completes the thesis.

SECTION II.

Part A. ERRORS IN THE DETERMINATION OF THE URINARY PREGNANEDIOL EXCRETION.

This section presents the results of a study of the variations found in the pregnanediol excretion in late pregnancy when a detailed examination of two methods of assay was made, together with a brief review of previous clinical experiences with these methods in the light of the results obtained.

INTRODUCTION.

Marrian isolated pregnanediol from the urine of pregnant women in 1929 and the chemical constitution was determined by Butenandt (1930 & 1931) who named the substance pregnanediol. Progesterone was isolated as a chemically pure substance in 1934. There is a striking relationship between these steroids and Venning & Browne (1936) demonstrated that sodium pregnanediol glycuronide could be recovered from the urine in the second half of the menstrual cycle and in pregnancy. Venning, Henry & Browne (1937) showed that when progesterone was injected into post-menopausal women and men pregnanediol was

recovered from the urine.

Pregnanediol can either be estimated as "free pregnanediol" or in the conjugated form, sodium pregnanediol glycuronide /Na P.G./. In the method used by Venning (1937) the /Na P.G./ is recovered from the urine by extraction with butyl alcohol and subsequent precipitation from acetone. This method was later modified by Allan & Viergiver (1941) and Kaufman & Westphal (1947). It requires large quantities of urine and the final extract contains only 80 per cent of the /Na P.G./ extracted,(Marrian & Gough, 1946). Moreover, bacterial hydrolysis of the /Na P.G./ sometimes occurs and this causes misleading low recoveries, (Bucher & Geschicter, 1940).

Astwood & Jones (1941) hydrolysed the urine by boiling with acid and estimated the "free pregnanediol" gravimetrically. "Free pregnanediol" when treated with concentrated sulphuric acid gives a yellow colour, and Talbot, Berman, Maclaglan & Wolfe (1941) used this to devise a colorimetric method for estimating the pregnanediol.

Sommerville, Gough & Marrian (1948) increased the purity of the final extract by the introduction of fractional precipitation of the pregnanediol extract. Modified precipitation techniques are used in the shorter methods of Guterman (1944) and of Sommerville, Marrian & Kellar (1948) but both these modifications reduce the accuracy and the specificity of the extraction process.

Other methods purify the pregnanediol by chromatography, Stimel, Randolf & Conn (1952), Huber (1947) and de Watteville (1951), but impurities are still present in their final extracts. These may be removed by washing with cold solvents, but this causes a considerable loss of the free pregnanediol. (Bradshaw & Jessop, 1953, and Klopper, Michie & Browne 1955).

When "free pregnanediol" is estimated, the final measurement is made either by a non-specific colour reaction obtained by adding concentrated sulphuric acid to the dried extract or by weighing it. The accuracy of the gravimetric method is dependent upon the purity of the residue and this may be determined by comparison of the melting

point and the absorption in ultraviolet light of the final extract with those of pure pregnanediol. These considerations led to the selection of the method of Sommerville, Gough & Marrian (1948) and to that of Henderson, Maclaghan, Wheatley & Wilkinson (1949), because they gave a white crystalline extract, and no loss was entailed by leaching the extract with cold solvents to purify.

DESIGN OF EXPERIMENT.

Three consecutive 24 hour specimens of urine were collected from 10 normal primigravid patients in the 34th - 35th week of pregnancy. Every care was taken to ensure a complete 24 hour specimen in each case; also the volume and creatinine content of each specimen of urine were noted and where these showed a marked daily variation the specimens were discarded and others obtained: this was only necessary on 2 occasions. Purified extracts were prepared in duplicate by myself and a technician. One of us used the method of Sommerville et al (1948) and the other used that of Henderson et al (1949). The pregnanediol content was estimated gravimetrically

and colorimetrically on each specimen of urine by both methods. The gravimetric extracts were also examined for purity by melting point determinations and absorption in ultraviolet light. Each patient was kept under observation until delivery when the weight of the baby, and the weight, macroscopic appearance and histology of the placenta were noted.

METHOD I.

The method of Sommerville et al (1948) was used without major modification. The urine (500 ml.) after acid hydrolysis, was extracted with toluene, and any emulsion obtained was broken down by the addition of a few drops of Teepol (Shell Chemicals, Ltd., London) instead of the tedious filtration process used by Sommerville and his colleagues; this modification was shown not to interfere with the extraction. The toluene extract was washed with N-NaOH and water and evaporated to dryness; the residue was then dissolved in ethanol and precipitated under carefully controlled conditions of time and temperature, once with 0.1 N-NaOH and twice with water, the precipitate in each case being controlled

by centrifugation with the aid of Hyflo Super-Cell (Johns Manville Co.,Ltd., London). After final decolorization with charcoal in ethanol, concentrated sulphuric acid was added to a portion of the dried extract which was then placed for 24 minutes in a water bath at 25°C; the absorption of the solution was then measured in the Spekker Photoelectric Absorptiometer using an Ilford Spectrum Violet 601 Filter (transmission max. 403 mμ). The remainder of the dried extract was weighed to give an alternative method of estimation.

METHOD II.

Henderson et al (1949) used a precipitation from acetone because this solvent appeared, from preliminary work, to give the best results. They showed that the time and temperature of precipitation from acetone were not determining factors in the recovery of added pregnanediol, and, as pregnanediol is less soluble in acetone than in ethanol, recovery from acetone should compare favourably with that obtained by the method of Sommerville et al (1948).

In the original method Henderson et al (1949) used only one precipitation; for Method II, the two

techniques have been combined, in that three precipitations from acetone instead of ethanol were used.

The dried toluene residue obtained as for Method I was taken up in 2.5 ml. acetone in a 50 ml. centrifuge and 50 ml. 1N-NaOH was added. The mixture was stirred and placed in a boiling water bath for 10 minutes and subsequently transferred to a refrigerator at 4°C for 2 hours. Hyflo Super-Cel 10 mg. was then added, with stirring, and the tube was centrifuged at 2,500 r.p.m. for 20 minutes. The supernatant liquid was decanted and the precipitation twice repeated on the residue using distilled water instead of 0.1 N-NaOH. The remainder of the procedure was as for Method I.

Recovery experiments were carried out for comparison with Method I. One precipitation using pure pregnanediol, pure solvents and 0.1 N-NaOH or distilled water, gave similar results for both methods; but when pure pregnanediol was added to male urine (0.5 - 2.0 mg. added to 500 ml. of urine) the recoveries using each technique in full

Table I. EXCRETION LEVELS OF PREGNANEDIOL IN mgms/24 hours.

Patients		DAY 1.		DAY 11.		DAY 111.		TOTALS	
		By Colour	By weight	By Colour	By Weight	By Colour	By Weight		
1	Method 1	45	93	27	65	31	56	542	
	Method 2	36	56	29	44	25	35		
2	Method 1	35	58	39	54	41	60	445	
	Method 2	17	33	23	37	21	27		
3	Method 1	37	64	50	79	56	89	512	
	Method 2	20	20	13	18	32	34		Method 1. 3641
4	Method 1	55	100	61	115	53	81	671	
	Method 2	34	43	32	42	25	30		Method 2. 1996
5	Method 1	53	59	54	63	52	61	503	
	Method 2	29	48	21	24	17	22		
6	Method 1	80	82	72	67	70	83	707	
	Method 2	34	70	10	57	12	70		
7	Method 1	48	64	42	53	44	57	451	
	Method 2	31	36	21	20	10	25		Colorimetric 2374
8	Method 1	80	76	69	48	68	72	741	
	Method 2	48	66	47	64	35	68		Gravimetric 3264
9	Method 1	48	49	64	48	52	46	449	
	Method 2	25	25	22	26	21	23		
10	Method 1	61	71	47	55	64	75	616	
	Method 2	33	55	22	54	31	48		
	Totals	2017		1798		1822		5637	

TABLE I

ANALYSIS OF VARIANCE

	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio	Probability
Between Days	721	2	360	2.7	0.1
Between Patients	9,416	9	1,046	7.8	Very small
Between Methods	22,550	1	22,550	170	Very small
Between colorimetric and Gravimetric Assay	6,586	1	6,586	50	Very small
Error	14,076	106	133		
Total	53,349	119			

were approximately 10% less for Method II than for Method I: this discrepancy was greater when pregnancy urine was examined. As the final product from pregnancy urine using either method was not pure pregnanediol, this discrepancy could be due to a difference in the amount of impurity present.

RESULTS OF THE EXPERIMENT.

The duplicate readings were first examined and the differences between pairs of readings analysed. The mean difference of duplicate readings in Methods I and II was 3.9 ± 0.57 (standard error of mean of 30 observations) and 2.5 ± 0.38 (30) showing that the technical accuracy of the two methods was similar. The duplicate readings were therefore averaged leaving 4 estimations of pregnanediol for each of the 10 patients on each of 3 days, making 120 estimations in all (Table I).

The marginal totals shown in Table I indicate the main results of the experiment. There is a large difference between individual patients and a small difference (not significant) between days, which may be ignored in view of the other and more

TABLE II

MELTING POINTS AND ULTRA VIOLET LIGHT ABSORPTION OF PURIFIED EXTRACTS

	2nd Product Method 1.			2nd Product Method 2.			Pregnanediol
	Mean	Standard Error	No. of Estimations	Mean	Standard Error	No. of Estimations	
Melting points	207	± 1.75	32	219	± 2.2	41	243
$E^{1\%}_{1cm.}$ at 204 mμ.	41.2	± 2.7	30	56.6	± 5.9	30	0.15
$E^{1\%}_{1cm.}$ at 230 mμ.	27.9	± 2.2	30	32.0	± 3.9	30	0.30

important findings. There are marked differences between the results for Method I and for Method II and between the gravimetric and colorimetric estimations, differences which are far greater than could possibly be the result of chance variation. These observations, together with the high mean square error, suggested that all four estimates were at fault, and this was shown to be the case when the final products were tested for purity by measuring their melting point and ultraviolet absorption spectra (Table II).

The average melting point for the product of Method I was 207° ± 1.8° (standard error of the mean of 32 observations), and for Method II 219° ± 2.2°(41), levels which were clearly different from those of pure pregnanediol (243°), all melting points being measured on a Gallencamp (London) stage. Though Method I gave estimations for pregnanediol twice those for Method II (see totals - Table I), the higher melting point of the final extract of Method II indicated that this method gave the purer products.

Table III

Case No.	Duration of Pregnancy at delivery	Babies' Weight	Placental Weight	Macroscopic appearance of Placenta
1	41 weeks	8 lb. 15oz.	1 lb. 14oz.	Few small infarcts present
2 *	39½ "	6 lb. 3 oz.	1 lb. 3oz.	Unhealthy "gritty" appearance. Long cord. No infarcts.
3	41 "	8 lb. 1 oz.	1 lb. 5oz.	Few small infarcts present.
4	43 "	8 lb. 1 oz.	1 lb. 8oz.	"Gritty" appearance. Few small infarcts.
5	40 "	4 lb. 9 oz.	1 lb. 2oz.	Small but healthy.
6	40 "	8 lb. 6 oz.	1 lb. 8oz.	Healthy.
7 *	44 "	9 lb. 7 oz.	1 lb. 12oz.	Unhealthy "gritty" appearance.
8	38½ "	6 lb. 13oz.	1 lb. 11oz.	Healthy. Few small infarcts.
9	41 "	6 lb. 12oz.	1 lb. 3oz.	Healthy.
10	40 "	7 lb. 5oz.	1 lb. 5oz.	Healthy with few small infarcts.

* In case 2 there was marked foetal distress but a living child was obtained.

* In case 7 the infant was stillborn.

The ultraviolet absorption spectra were measured in ethanolic solution and peaks or inflections were obtained in all cases at approximately 204 and 230 mu. The average values for $E^{1\%}_{1\ cm.}$ for Methods I and II at 204 mμ. were 41 ± 2.7 (30) and 57 ± 5.9 (30) and at 203 mμ. the corresponding values were 28 ± 2.2 (30) and 32 ± 3.9 (30). As pregnanediol at the concentration and within the range investigated has a negligable absorption, these peaks must have been due to impurities and the absorption measured at these wavelengths is an indication of some of the impurity present.

The large difference between colorimetric and gravimetric estimation (see totals Table I) stresses the impurity of the product and the non-specificity of the method of assay.

STUDY OF PLACENTAE.

The relationship between the duration of pregnancy and Baby's weights are shown on Table III. There was no correlation found between the pregnanediol excretion, by either method and these results.

Nor was there any correlation between the histological appearances of the placentae and the pregnanediol assays.

REVIEW OF PREVIOUS CLINICAL EXPERIENCE WHEN THE METHOD OF HENDERSON ET AL (1949) WAS USED.

The records of 24 gynaecological cases were available for study; infrequent or irregular menstruction or infertility were the main complaints. In all cases pregnanediol was estimated by Method II. Nine of these patients had levels ranging from 0.7 - 4.5 mg./ 24 hours from the 14th to 28th day of the menstrual cycle, and in all cases the endometrium showed histological evidence of progesterone activity. In 2 other cases, although pregnanediol was detected in the urine, the endometrium was of the hypoplastic non-secretory type. In the remaining patients, evidence of ovarian or pituitary dysfunction was detected by other endocrine assays, and no pregnanediol was present in the urine.

Only 2 abnormally high levels of pregnanediol excretion were found among 700 patients investigated, both occurring in hermaphrodite children aged 4

Table 4.

THE RESULTS OF THE ENDOCRINE INVESTIGATION OF TWO HERMAPHRODITE CHILDREN.

		Case 1.	Case 11.
Colorimetric Estimation of Pregnanediol	Method 1.	79	24
	Method 2.	51	6.4
Gravimetric Estimation of Pregnanediol	Method 1.	130	-
	Method 2.	-	29
1% $E^{1cm.}$ at 235 mμ.	Method 1.	80.7	-
	Method 2.	-	71.5
17 Ketosteroid Excretion mgms/day		45	11

years. The final pregnanediol fraction consisted in each case of a brown oily substance instead of white crystalline solid. The results are shown in Table IV. The ultraviolet absorption spectra showed a marked peak with a very high extinction value in both cases at 236 mμ., thus indicating the possible presence of a large quantity of unsaturated ketone. Cortisone therapy (50 mg./day intramuscularly) reduced the level of excretion of this material to zero within 4 days, indicating that the substance was probably of adrenal origin. Thus false positive pregnanediol excretions were found in these cases.

The individual records of 20 cases of habitual or threatened abortions were also available for close study. Not all cases where low levels of pregnanediol were detected, miscarried, and in some cases with normal levels the outcome from pregnancy was unsatisfactory. Weekly determinations were carried out in one patient with a history of 2 previous miscarriages. She had stilboestrol and progesterone therapy during the investigation and delivered a living child at term;

the pregnanediol excretion in this case varied markedly from week to week.

In a few cases of pre-eclamptic toxaemia where an attempt had been made to use the estimation as a measure of placental function, marked variation in the results obtained rendered this impossible. Thus in 2 cases where the patient delivered living babies at 34/35 weeks the urinary pregnanediol excretion levels were 5.6 and 6.5 mg. respectively.

DISCUSSION.

The work reported here shows clearly that the estimation of urinary pregnanediol by the above techniques, which were chosen as being the most satisfactory available, must be of very doubtful clinical value. Considerable variation has been shown in the level of excretion of pregnanediol in normal pregnancy between individuals and possible a small day to day variation in the same individuals. A marked variation was apparent when comparisons were made between the

two methods of obtaining the purified extract and between the two methods of estimating the pregnanediol content. There was also a high error variation. The melting points and absorption spectra of the end products together with the difference in values obtained by colorimetric and gravimetric estimation indicate that much of this variation is due to the presence of impurity. This has been proved in the past to give fictitiously high results for most of the shorter methods for estimating pregnanediol based on the procedures of Astwood & Jones (1941) and Talbot, Berman, Maclaghan & Wolfe (1941).

In the light of these errors a careful re-assessment of previous experience with these methods of assay was undertaken lest any useful clinical correlation be overlooked. None was found. The variation in the results in cases of habitual and threatened abortion and pre-eclamptic toxaemia made it impossible for the test to be used as a diagnostic aid in cases cited.

False positive results for pregnanediol were obtained in 2 gynaecological cases in which the

endometrium was of the hypoplastic non-secretory type and in 2 cases of hermaphroditism in children. These observations are in line with that of Swyer (1949) who stated "The total excretion of pregnanediol in the luteal phase of the menstrual cycle was so variable that one cannot hope to give indisputable evidence of corpus luteal activity".

SUMMARY AND CONCLUSIONS.

The levels of urinary pregnanediol excretion in late pregnancy were studied by two methods of assay.

In a planned study of the variations in these levels, estimations were made on the 24-hour urine specimen of each of 10 normal 34-36 week primigravid women on each of three consecutive days. Purified extracts from each urine sample were prepared by each method and the pregnanediol content estimated both gravimetrically and colorimetrically.

The 4 methods of assay gave widely different results which were analysed by the analysis of variance.

The difference between the results obtained by the two methods and by the gravimetric and colorimetric assay were highly significant.

The melting points and the ultraviolet light absorption of the purified extracts were also investigated and were found not to be those of pure pregnanediol.

It was concluded that the impurities present in the purified extracts accounted for much of the variation in the results obtained and for the high level of uncontrollable error.

A review, to determine any clinical value of these assays, carried out over several years, did not reveal any clear evidence of usefulness which might be set against the errors above described.

It was thus concluded that these methods were not sufficiently specific, sensitive or accurate for the estimation of urinary pregnanediol, and that further research upon the method of extraction would be necessary before any study of the pregnanediol excretion in abnormal pregnancy could be contemplated.

Part B.

ANALYSIS OF THE METHOD FINALLY CHOSEN FOR ESTIMATING THE URINARY PREGNANEDIOL.

INTRODUCTION.

A new method of estimating pregnanediol was reported by Klopper, Michie & Brown (1955). Through the courtesy of Dr. Klopper, the details of this method were made available to me before publication and an experiment, similar in design to that previously employed, was undertaken to evaluate this new method.

DESIGN OF EXPERIMENT.

Three consecutive carefully collected 24 hour specimens of urine from each of 10 normal patients in the 32 - 36th week of pregnancy were examined. The pregnanediol content was estimated independently by myself and a technician, duplicate estimations being carried out on the same specimen of urine.

METHOD.

The only major modification to the method of Klopper et al (1955) was the omission of the

permanganate wash. Recent work by Klopper (1955), completed after we had begun this investigation, indicated that this step is necessary in the case of non-pregnant urines and although desirable in pregnancy urines, is not essential in the majority of cases. (Klopper 1955) .

In 5 cases (1 - 5 Table I) duplicate samples of urine were taken, each being one twentieth of the 24 hour volume; these provided sufficient material for the tests for purity on the final extract in addition to the normal assay. In the other 5 cases (6 - 10 Table I) pregnanediol assays only were carried out and a smaller quantity of urine, (1/250th) was taken. After acid hydrolysis the urine was extracted with toluene and this extract washed first with N-NaOH and then with distilled water, and evaporated to approximately 10 ml. This was applied, after cooling to room temperature, to the surface of a chromatography column previously prepared from 3 g alumina in a tube of 1 cm. diameter. The column was washed with 25 ml. 0.8% ethanol in benzene, and the pregnanediol fraction then eluted

with 13 ml. 3% ethanol in benzene. (The volumes of solvents used in chromatography have to be slightly modified with different batches of alumina). The second eluate was evaporated to dryness and the residues acetylated with acetyl chloride in benzene, 2 ml. of each. Light petroleum, (b.p. 40° - 60°) was then added, and the solution washed with 25 ml. 80% strength aqueous bicarbonate, and twice with 25 ml. distilled water and chromatography was again carried out. The column was similar to that used previously but it was prepared in light petroleum instead of benzene. The pregnanediol diacetate was eluted with 15 ml. of benzene. This was evaporated to dryness and approximately 10 mg. sodium sulphite was added. The colour was developed by adding 10 ml. of concentrated sulphuric acid and allowing the solution to stand in a water bath at 25° for 1 hour, the absorption of the solution was measured in the Spekker Photoelectric Absorptiometer using an Ilford Spectrum Violet 601 filter, (transmission maximum 403 mμ.).

When the twentieth of the 24-hour specimen was

Table I. MELTING POINTS AND ULTRAVIOLET ABSORPTION OF PURIFIED EXTRACTS.

Case No.	MELTING POINT °C			UV ABSORPTION OF FINAL PRODUCTS IN ETHANOL $E^{1\%}_{1\ cm}$ at 210 mu.			UV ABSORPTION OF FINAL PRODUCTS IN H_2SO_4 $E^{1\%}_{1\ cm}$ at 285 mμ.			* $E^{1\%}_{1\ cm}$ at 420 mμ.		
	1	2	3	1	2	3	1	2	3	1	2	3
1	157	157	158	12.6	11.3	12.5	271	324	392	170	180	168
2	163	162	161	11.3	11.3	12.2	212	293	206	135	140	117
3	157	159	156	23.0	25.2	21.2	460	464	385	236	147	243
4	159	160	157	22.8	19.0	11.2	418	356	405	177	184	167
5	156	156	155	9.3	16.4	12.1	244	270	155	195	214	171
Pure Ø Pregnanediol di-acetate	163 - 179 Bi-phasic				3.9			293			155	

* The percentage concentration is based on assay as pregnanediol by the H_2SO_4 colorimetric technique.

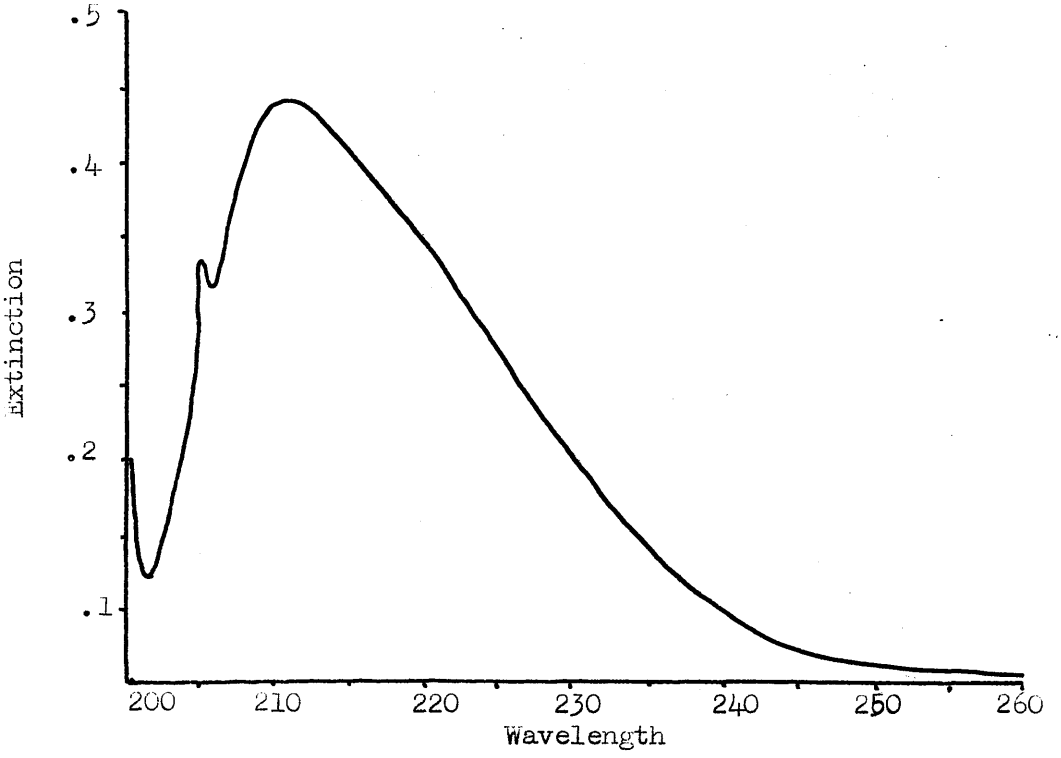
Ø Produced by acetylation of pregnanediol (M.P. 243°) followed by chromatography and repeated re crystalisation from di-ethyl ether.

extracted, the final products were examined for purity by measuring their melting points on a Gallencamp (London) stage and their ultraviolet absorption spectra both in ethanol and sulphuric acid in a Unicam (Cambridge) Quartz Spectrophotometer, S.P.500.

Recovery experiments were carried out by adding 0.2 mg. pure pregnanediol in 0.2 ml ethanol to 150 ml. male urine or 10 ml. late pregnancy urine, followed by hydrolysis and extraction, as previously described. Four male urines and two late pregnancy were studied.

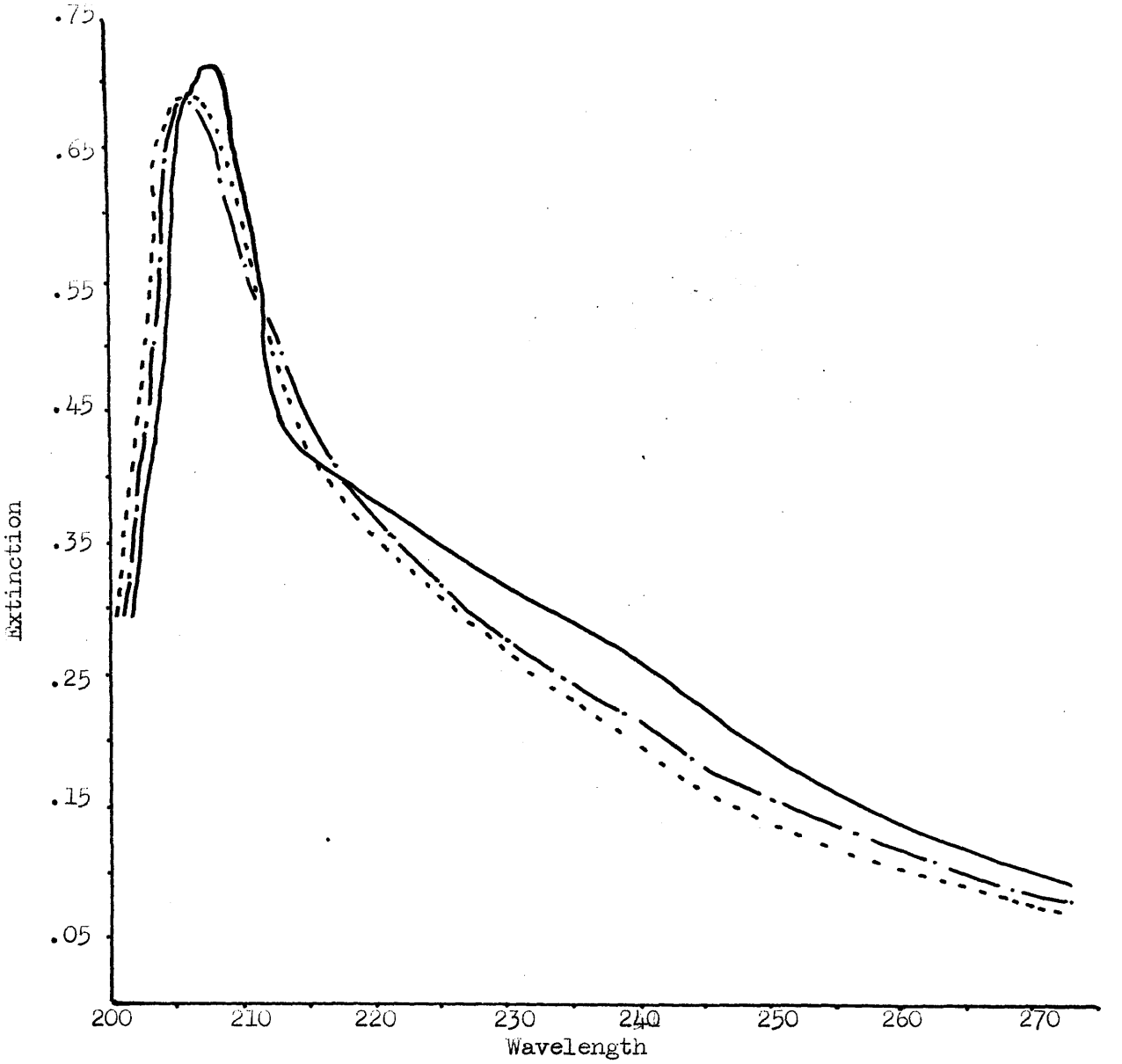
RESULTS.

The melting points of the final acetylated products in the case of patients 1 - 5 are shown in Table I. The melting points were bi-phasic, but only the lower temperatures are shown. The ultraviolet absorption was measured in ethanolic solution, and after 1 hour in concentrated sulphuric acid at 25° C. The peaks in all cases were in the same positions as those for pure pregnanediol di-acetate but the extinction values of these wavelengths were



Graph I a. THE ULTRAVIOLET ABSORPTION CURVE FOR PURE PREGNANEDIOL DI-ACETATE IN ETHANOL IN 1 mg./ml. ETHANOL.

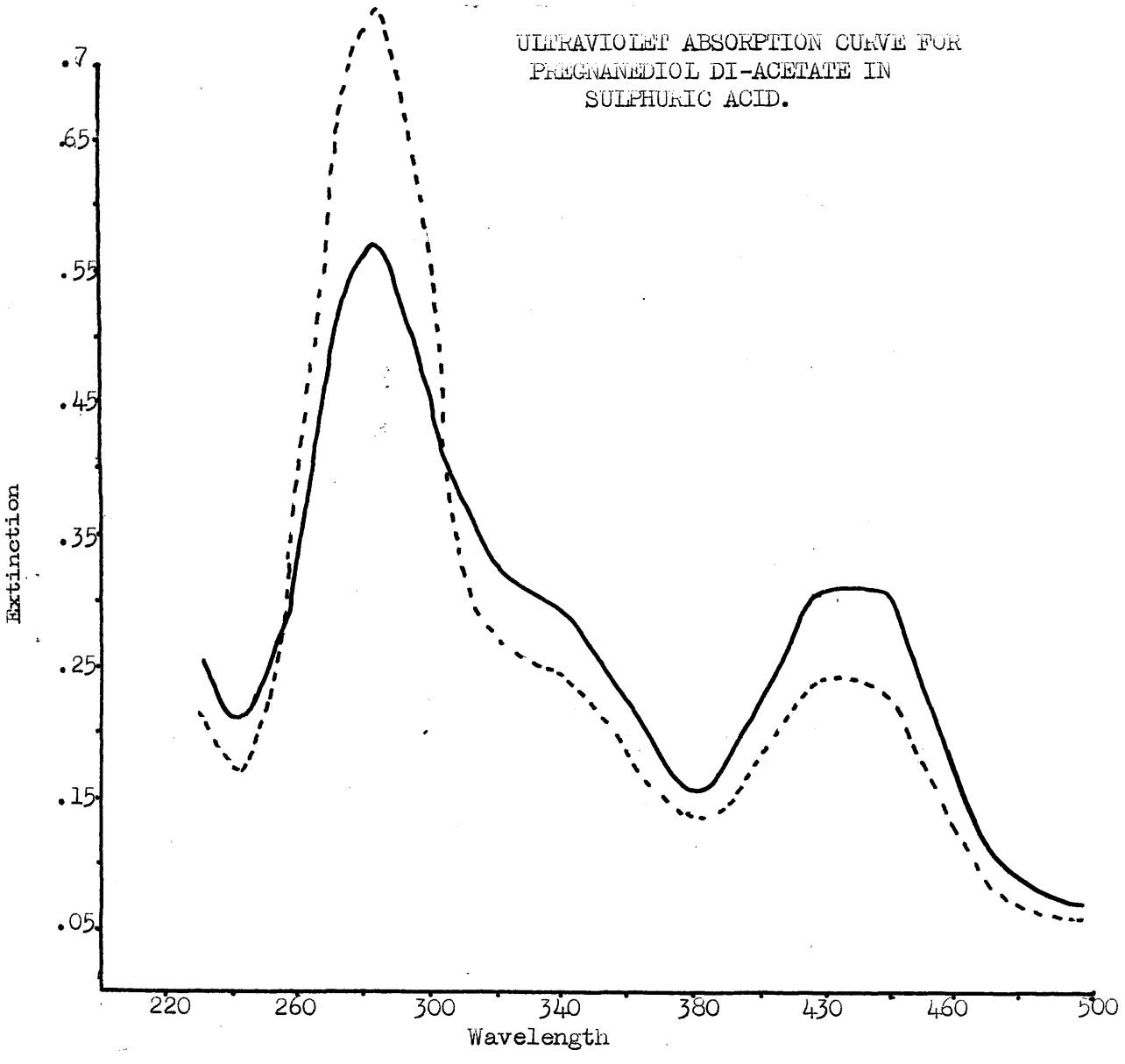
E. 210 = .390 $E^{1\%}_{1cm.}$ 390



Graph I.b. ULTRAVIOLET ABSORPTION CURVE FOR PREGNANEDIOL DI-ACETATE IN ETHANOL (EXTRACT) FROM CASE No. 1.

		E. 210 mμ./ml.	$E^{1\%}_{1cm.}$
———	Day I	.454	12.6
- - - - - -	Day II	.500	11.3
.———.	Day III	.456	12.5

Graph II a.



——— Pregnanediol di-acetate (prepared by Dr. Klopper)

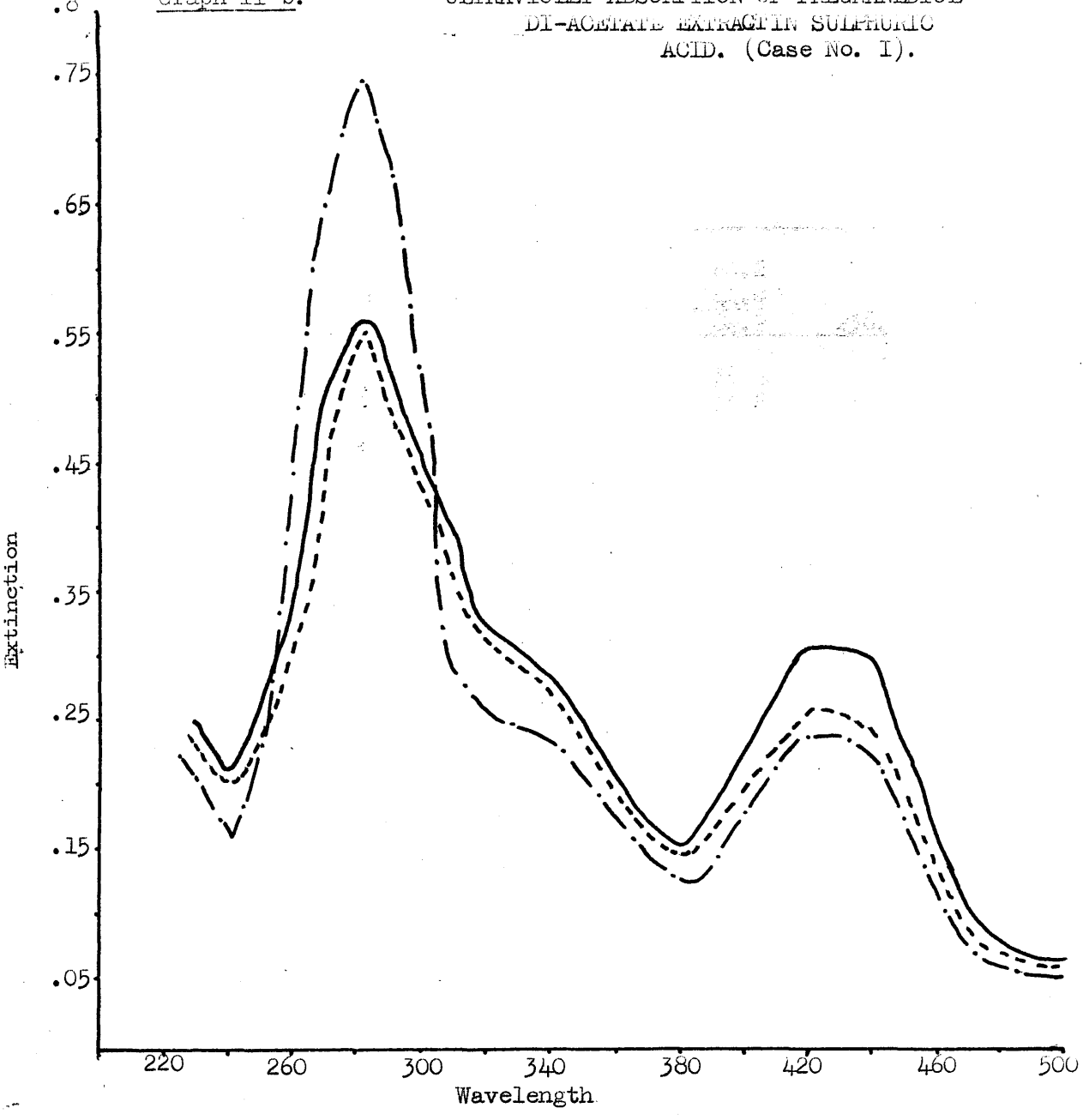
– – – – – Pregnanediol di-acetate (prepared by J.H.W. Staff).

207µg./ml H_2SO_4. 1%

$E^{1\%}_{1cm}$	285 mµ.	(K) .758	293	1cm.
		(J.H.W.) .585	379	
$E^{1\%}_{1cm}$	420 mµ.	(K) .244	155	
		(J.H.W.) .310	122	

Graph II b.

ULTRAVIOLET ABSORPTION OF PREGNANEDIOL DI-ACETATE EXTRACTIN SULPHURIC ACID. (Case No. I).



	E. 285 mμ./ml	E 1% 1cm.	E. 420 mμ./ml	E 1% 1cm.
——— Day I	.55	211.5	.35	134.6
- - - - - Day II	.76	292.5	.365	140.2
·——· Day III	.565	205.5	.315	116.7

TABLE II

EXCRETION OF PREGNANEDIOL IN MGMS. PER 24-HOURS ON THREE CONSECUTIVE DAYS IN NORMAL 32 - 36 WEEK PREGNANCIES

Patients	Reading	Day 1	Day 2	Day 3	Sum, 6 Readings	Average Reading
1	1	39.5	36.6	40.2		
	2	40.0	35.8	44.0		
	Sum	79.5	72.4	84.2	236.1	39.35
2	1	31.6	34.8	38.4		
	2	27.8	33.8	35.8		
	Sum	59.4	68.6	74.2	202.2	33.70
3	1	51.5	51.9	53.2		
	2	51.8	52.8	52.3		
	Sum	103.3	104.7	105.5	313.5	52.25
4	1	36.3	40.0	37.5		
	2	36.9	37.5	36.7		
	Sum	73.2	77.5	74.2	224.9	37.48
5	1	35.6	38.2	35.8		
	2	34.8	36.2	36.6		
	Sum	70.4	74.4	72.4	217.2	36.20
6	1	44.8	48.0	48.3		
	2	48.7	46.3	52.1		
	Sum	93.5	94.3	100.4	288.2	48.03
7	1	26.6	29.6	23.0		
	2	26.8	28.5	23.9		
	Sum	53.4	58.1	46.9	158.4	26.40
8	1	27.3	25.3	26.8		
	2	25.3	25.8	26.3		
	Sum	52.6	51.1	53.1	156.8	26.13
9	1	27.1	27.0	26.9		
	2	31.3	25.8	26.8		
	Sum	58.4	52.8	53.7	164.9	27.48
10	1	41.2	41.3	41.9		
	2	43.2	42.7	39.2		
	Sum	84.4	84.0	81.1	249.5	41.58
SUM, 20 READINGS.		728.1	737.9	745.7	2211.7	
AVERAGE READING		36.41	36.90	37.29		36.86

invariably increased (Graph I & II).

The final extract was thus purer than that which was obtained by the methods which had been previously tested.

The 24-hour pregnanediol excretions were analysed statistically. After averaging the duplicate readings, the results were set out in table form, (Table II), where the marginal totals reveal the main features of the experiment. Using the analysis of variance the patients differed significantly, as was expected. The interaction Patients X days is significant, indicating that even when allowance is made for the testing error (deduced from comparison of the readings of the two observers) the estimated excretion in any particular patient does not vary from day to day. This "occasion" variation has an estimated standard deviation of $\sqrt{\frac{8.5 - 2.1}{2}}$ i.e. 1.8 appears to be random, the lack of significance between day mean square suggesting the absence of any appreciable daily pattern of variation common to all patients. The testing error of a single sample has a standard

Table III.

ANALYSIS OF VARIANCE

Source of Variation.	Degrees of Freedom	Sum of Squares	Mean Square
Between women	9	4280	475.6 xxx
Between days	2	8	4.8 ns
Interaction or Random error	18	153	8.5 xxx
Residual or Testing error	30	62	2.10
Total	59	4503	-

The mean squared difference between women is significant on the 0.1% level. The difference between days is not significant when compared with the interaction mean square which forms the appropriate basis for such a test. The interaction mean square itself is significant on 0.1% level when tested against the testing error mean square.

deviation of approximately 1.45. Hence if any attempt is made to measure the general level of output at any particular phase of pregnancy within the period covered by the experiment using only one test by one person the error will be the sum of the occasion variation and the testing error and will have an estimated standard deviation $(1.45)^2 + (1.8)^2$ i.e. 2.30.

DISCUSSION.

In the critical evaluation of this new method of assay, some uncertainties need to be discussed.

The specificity of this method, as with the older techniques previously studied, depends upon the purity of the final extract. Using the melting points and ultraviolet absorptions as criteria of purity, it has been shown that a final product is obtained which is only slightly less pure than a specimen of pregnanediol-di-acetate, produced by acetylation of a pure sample of pregnanediol followed by chromatography and repeated recrystalisations from ether.

Recovery experiments when pure pregnanediol was added to the urine show that 80-96% of the pregnanediol originally present in the urine is estimated in the final extract. These findings are similar to those reported by Klopper et al (1955).

The laboratory error of this method was determined when the assay was performed on three consecutive 24-hour specimens of late pregnancy urine. Good duplication of the results by independent observers shows that the laboratory error is low; however with pregnancy urine it is necessary to use only a small portion, (1/250), of the 24-hour specimen and thus any laboratory errors are multiplied by 250 when the 24-hour output is estimated. Despite this, the standard deviation of the testing error is only 1.49.

The random errors due to day to day variation in laboratory conditions, faulty urine collection, etc., have been reduced so far as could be contrived in the results reported here: but these known and unknown sources of variation can never be entirely eliminated. The best that can be hoped for is that

the residual error is lower than the changing levels which are sought. In this study the standard deviation for the residual error was encouragingly low 2.3 due in a large measure to co-operation of the patients selected. Five were doctors' wives and the other five were nursing sisters, all of whom were aware of the need for great care in collection complete 24-hour specimens of urine.

The evident superiority of the method of Klopper et al (1955), as compared with the previous techniques examined, thus provided a means for routine clinical investigations. Provided that great care is taken at all stages of the assay, particularly in the accurate collection of the twenty-four hour sample, results of reasonable dependency can be expected.

SUMMARY AND CONCLUSIONS.

The method developed by Klopper et al (1955), for estimating urinary pregnanediol has been investigated in late pregnancy, and the results reported here.

As a planned study of the method and of the variations in the daily excretion under known conditions, estimations were made on 24-hour specimens of each of 10 normal 34-36 week primigravid women on each of three consecutive days.

Measurement of the melting points and ultraviolet light absorption showed the final extract to have a high degree of purity.

The analysis of variance of the results showed a marked difference between the amounts of pregnanediol excreted by the different patients; but no difference between the daily outputs. The testing error of the method as estimated from the duplicate results of two independent observers was low. The residual or "occasion" error was small enough to justify a further study of the test.

This method was thus chosen to study the application of this assay in the management of early and late pregnancy disorders.

Part C.

THE RELATIONSHIP BETWEEN "PROGESTATIONAL ACTIVITY" OF THE BLOOD AND URINARY PREGNANEDIOL EXCRETION IN NORMAL PREGNANCY.

Hooker & Forbes (1947) described a technique whereby "progestational activity" could be measured by observing the response of the stromal cells of the endometrium of mice to local applications of progesterone or extracts containing substances of "progestational activity". Essentially the test is carried out by injecting measured micro-quantities (0.0006 ml. or less) of the fluid under test into the uterine horn of a previously ovariectomised mouse, leakage of the injected fluid being prevented by two ligatures one above and one below the injection mass. The characteristic response in the endometrial stromal cells, seen 48 hours after injection, consists of a change from stellate or polygonal cell forms to a smooth, slightly elongated oval outline; the chromatin threads become fine and evenly distributed, and the nucleolus is conspicuous.

The minimal dose of progesterone to produce this effect in the strain of mice employed by these workers was 0.0002 μ g. The authors showed that other steroids failed to produce this change and concluded that the test was reasonably specific in denoting progestational activity Forbes (1950) also produced evidence to show that progestational activity could be demonstrated in plasma extracts of as little as 1 ml. of blood taken from women during the secretory phase of the menstrual cycle.

Hitherto the biological test for progestational activity was carried out in rabbits and the amount of progesterone necessary to produce positive responses precluded the possibility of applying this test to the routine study of blood plasma levels of progesterone in women. With the Hooker & Forbes' Test available, it was decided to determine whether the progestational activity level, measured in terms of progesterone, bore any relationship to the level of pregnanediol in the urine. Preliminary tests, using solutions of progesterone in sesame oil, showed that with the

strain of mice available the sensitivity of the test was strictly comparable with that described by Hooker & Forbes (1947).

METHOD.

Three consecutive 24 hour specimens of urine were collected during the 34th to 36th week from each of two patients who subsequently were delivered of live babies. Pregnanediol estimations were carried out on each of these three specimens in each case, and the average daily excretion determined. On the second day of collection, a specimen of citrated blood was obtained. The citrated plasma was extracted twice with 10 ml. ether, and then 2 ml. sesame oil was shaken up with the ether extract. The ether was removed by distillation under reduced pressure, and 0.0006 ml. of the final solution or dilutions thereof were injected into the uterine horns of ovariectomised mice. The greatest dilution giving a positive response was considered to show progestational activity equivalent to 0.0002 u g. progesterone, the activity of the plasma then being expressed in terms of u g. progesterone per ml.

Table I. THE BLOOD "PROGESTATIONAL ACTIVITY" IN NORMAL PREGNANCY.

Case No.	Final duration of pregnancy in weeks	Duration at time of Estimation in weeks	Average Pregnane-diol excretion in mg/24 hrs.	"Progestational activity" in ug. Progester-one /ml.
1	41	34	26.2	3.4 - 8.5
2	38	35	26.6	> 8.5
3	40	33	27.5	3.4 - 8.5
4	40	34	32.2	3.4 - 8.5
5	39	36	36.5	0.9 - 1.7
6	41	34	37.3	0.2 - 0.9
7	40	34	39.3	0.2 - 0.9
8	41	32	42.1	> 8.5
9	40	35	48.0	0.9 - 1.7
10	40	34	52.0	1.7 - 3.4

No attempt was made to hydrolyse the plasma extract and therefore the recorded values are an expression of free progestational activity, and do not include any combined or "bound" progestins.

The results in the ten cases are set out in Table I.

The results show that whereas positive responses were obtained by the Hooker Forbes (1947) technique in all ten cases examined, there was no correlation between the level of response and the level of pregnanediol excretion. It seemed improbable that this technique would give an index of placental activity more reliable than the pregnanediol assay, and no further work using this technique was carried out.

SECTION II

THE PREGNANEDIOL EXCRETION IN NORMAL PREGNANCY AND IN THE PUERPERIUM.

The excretion of pregnanediol during pregnancy has been studied by many workers and the results show a general trend of high level excretion in the last three months of pregnancy with a fall to zero levels soom after delivery. Thus Browne, Henry & Venning (1937) estimating sodium pregnanediol glycuronide, obtained values of 40 mg./24 hours at the 21st week, peak values of 73 to 80 mg./24 hours at the 32nd week and showed that there was a fall just before delivery. Estimating "free pregnanediol" by precipitation techniques values of between 36 and 120 mg./24 hours were obtained in the later weeks of pregnancy by Davis & Fugo (1947), Guterman (1944) (1945), Jones, Delfs & Straun (1944) and Michie (1953). Lower values were obtained by de Watteville (1951) and Trolle (1955). The pattern of excretion immediately preceding parturition is not clear. Stover & Pratt (1939) and Hain (1941) found that the excretion was

maintained until the onset of labour. In contrast, a fall in excretion rate was demonstrated by several workers (Pratt, Simmonet & Robson, 1940, Lyon, 1940 and Maunsey, 1950).

Other workers were unable to demonstrate a characteristic excretion pattern for the period immediately preceding delivery (Venning, 1948, Bradshaw & Jessop, 1953, and Trolle, 1955). There was general agreement that pregnanediol falls rapidly after delivery and reaches low levels in 3 to 6 days.

Variations in the techniques of assay are in part clearly the cause of the lack of uniformity in the results reported by the above workers and accordingly it was necessary to study the pattern of pregnanediol excretion throughout normal pregnancy and the early puerperium by the method of Klopper et al (1954).

PLAN OF INVESTIGATION.

The following aspects of the pregnanediol excretion in normal pregnancy were studied:-

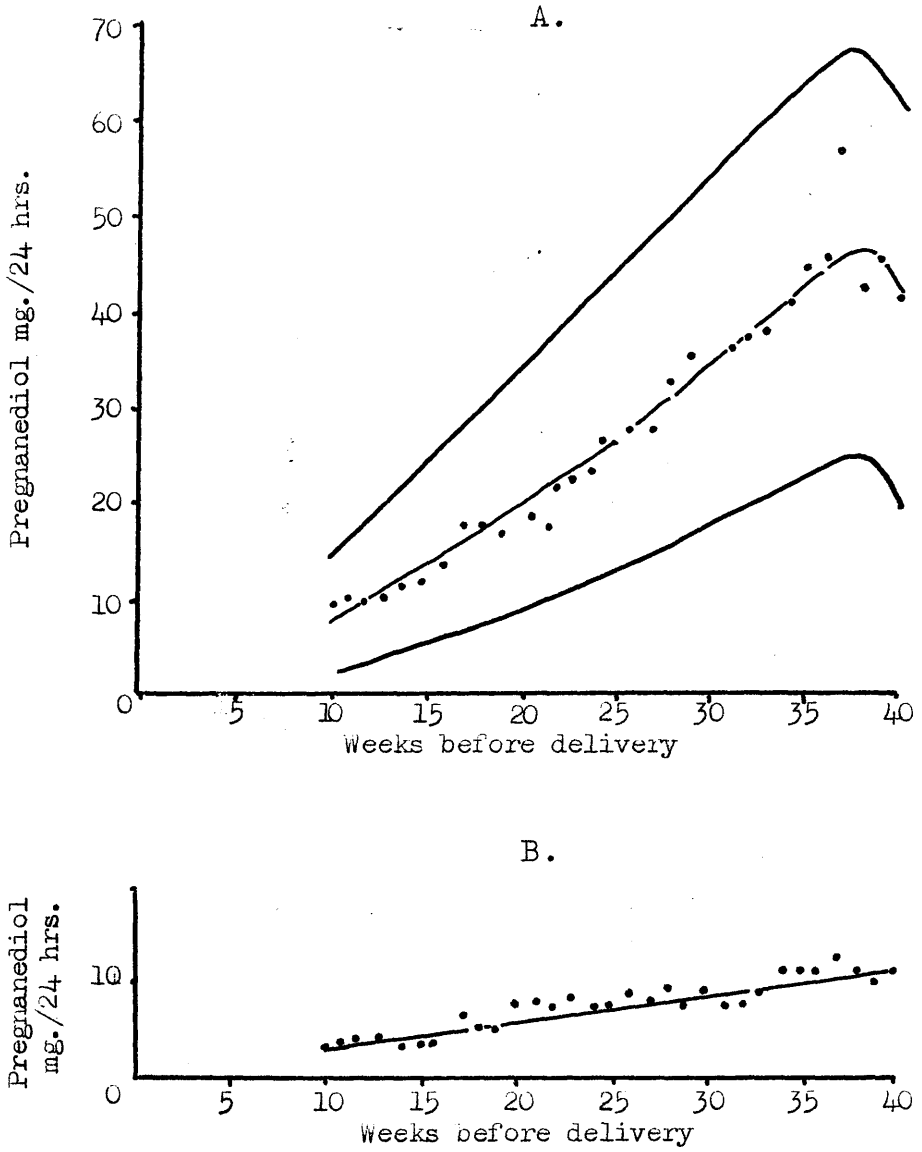


Fig. I.A. MEAN ESTIMATES OF URINARY PREGNANEDIOL EXCRETION FOR EACH WEEK OF PREGNANCY.

Fig. I.B. STANDARD DEVIATIONS CALCULATED FOR EACH WEEK OF PREGNANCY.

_____ Range of excretion ± 2 x S.D.....

__ __ Mean estimates.....

1) The pregnanediol excretion from the 10th week of pregnancy till the onset of labour.

Pregnanediol estimations were made on the urines of 24 women at intervals varying from 1 to 3 weeks throughout the course of pregnancy. They were a highly selected group of patients, being either the wives of doctors or former nursing sisters of the hospital. They all delivered living babies at the 38th to 42nd week of pregnancy and in each case the birth weight of the baby and the weight of the placentae was noted.

2) The pregnanediol excretion after parturition. Four hourly estimations were made in five cases during the 24 hours after delivery. All specimens were collected by catheter to prevent contamination with blood. In 9 cases pregnanediol determinations were made at "intervals" until the 9th or 10th day after delivery.

RESULTS.

The mean estimates of the urinary pregnanediol excretion during each week of pregnancy are shown on Fig.I. The range of excretion throughout normal

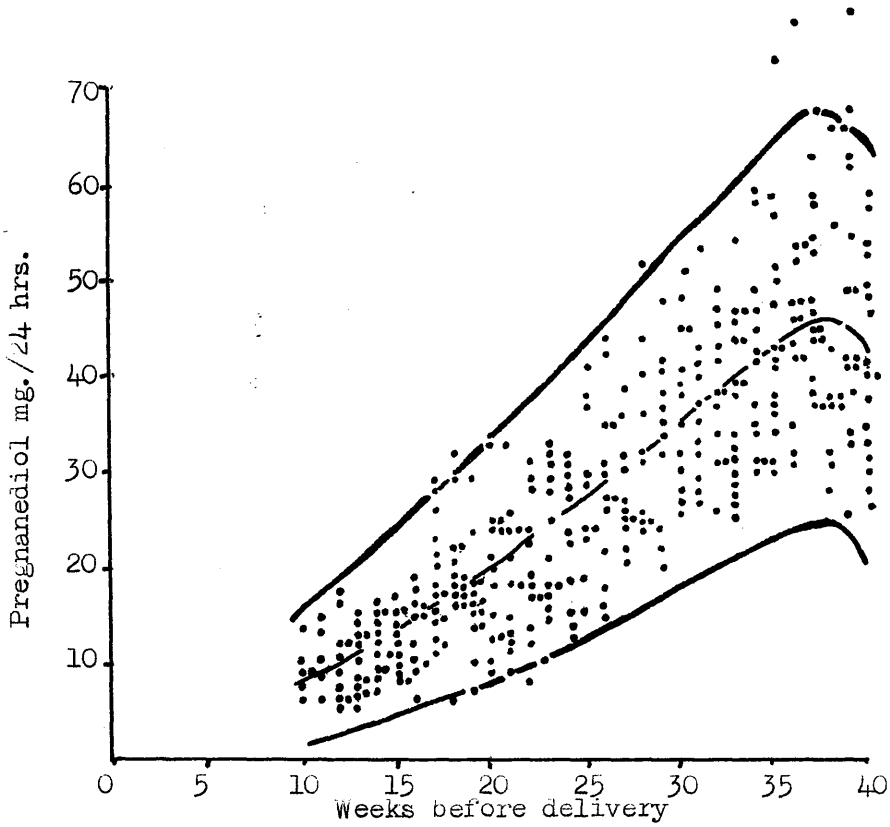


Fig. II. THE PREGNANEDIOL EXCRETION IN NORMAL PREGNANCY CALCULATED FROM THE DURATION OF TIME BEFORE DELIVERY.

——— Range of pregnanediol excretion.

— — — Average excretion.

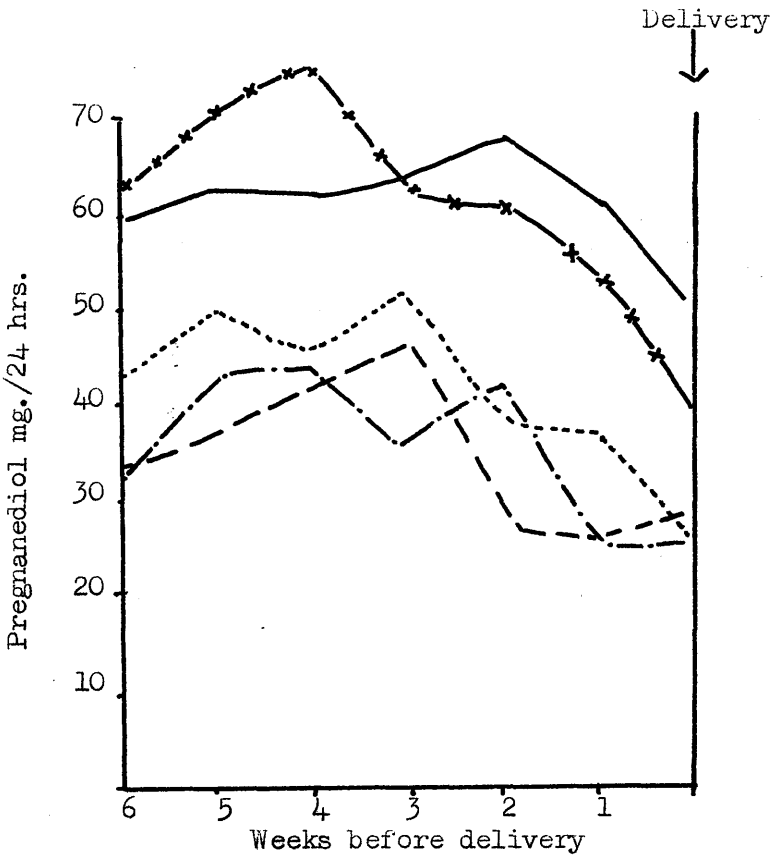


Fig. III. THE PREGNANEDIOL EXCRETION, IN 5 NORMAL PREGNANT WOMEN, DURING THE 6 WEEKS BEFORE DELIVERY.

In these cases there was a fall in excretion before delivery.

pregnancy is shown on Fig. II; it is a scatter diagram of all the readings obtained in the 24 cases, the mean and standard deviations being calculated for each week of pregnancy. Because the duration of pregnancy varied from 38 to 42 weeks the points on the diagram are plotted according to the levels of pregnanediol and the duration of time before delivery. It will be noted that the range calculated from the mean $\pm$ 2 S D varies from 2 to 15 mg./24 hours at the 10th week to 27 to 69 mg./24 hours at the 37th week the values falling to 20 to 64 mg./24 hours at the 40th week of pregnancy.

The trend of pregnanediol excretion in five cases where weekly determinations were made throughout the course of pregnancy is shown in Fig. III. Variations in the weekly excretion, in the same patient, occur but where a fall in the excretion is noted it is seldom more than 10 mg./24 hours and it is never below the normal pregnancy range. In the five cases shown in Fig. III the pregnanediol excretion fell during the 2 to 4 weeks before delivery.

Fig. IV shows the pregnanediol excretion in 4

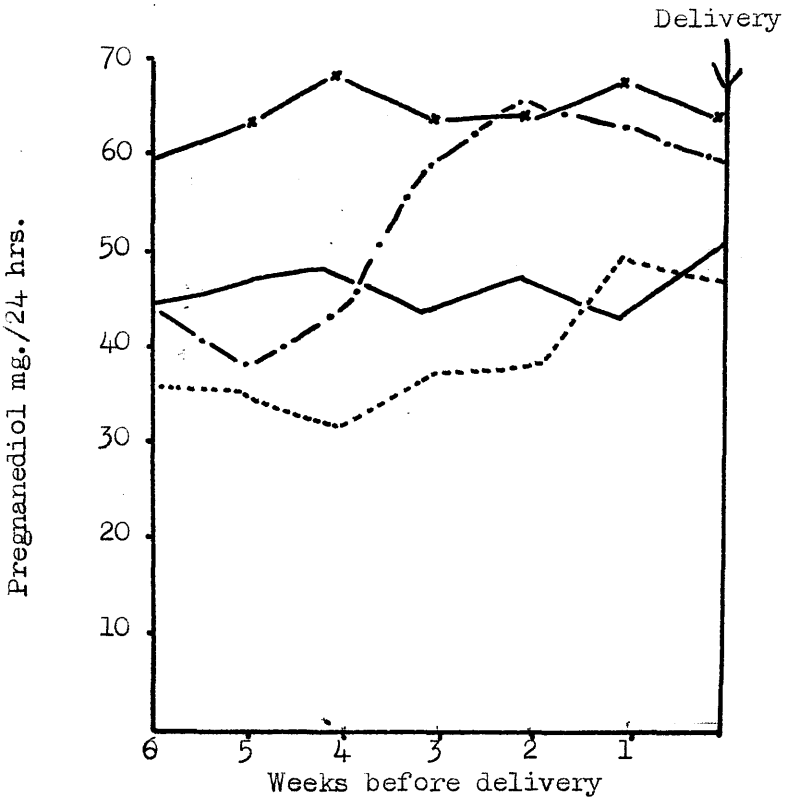


Fig. IV. THE PREGNANEDIOL EXCRETION, IN 5 NORMAL PREGNANT WOMEN, DURING THE 6 WEEKS BEFORE DELIVERY.

In these cases a fall before delivery was not detected.

Fig. V. THE DAILY PREGNANEDIOL EXCRETION IN 4 NORMAL PREGNANT WOMEN.

Table VI. THE PREGNANEDIOL EXCRETION BEFORE AND AFTER DELIVERY IN 9 NORMAL PREGNANT WOMEN.

Case No.	Days before delivery 5	4	3	2	1	Delivery	Days after delivery 1	2	3	4	5	6	7	8	9	10
1			28	26	28		15	16	8				5		3	
2	36	37	41	48	43		22	14	13			4.1				
3	25	25	26	25	22		15	7.4		6			4			4
4			62	60	59		29		8			5			3	
5		41		41	37		32	12			4			5		
6	38	41	38	43	39		27	8			5		3		2	
7			30		31		25	9		7		7		3		
8	45	41	40	43	43		41	15		3		3		4		2
9				40	40		35		9			2			2	

cases where a fall before the onset of labour was not detected. In one of these cases a rise in excretion was actually detected during the 2 weeks before delivery.

When daily estimations were made in 4 cases a constant excretion was maintained for 10 days in 3 cases and in one patient a rise occurred five days before delivery, Fig. V.

Fig. VI shows the pregnanediol excretion before and after delivery in 9 cases, it will be noted that there is a fairly constant daily excretion before delivery in all cases. During the first 24 hours after delivery the excretion is maintained at a high level. In 5 cases it is less than 6 mg./24 hours lower than the excretion before delivery. A rapid fall occurs during the next 10 days and levels of 2 to 4 mg./24 hours are found on the 10th day of the puerperium.

When four hourly estimations were made in five patients immediately after delivery, a high level of excretion was found in the first four hours (5 - 17 mg./ 4 hours). There was then a rapid

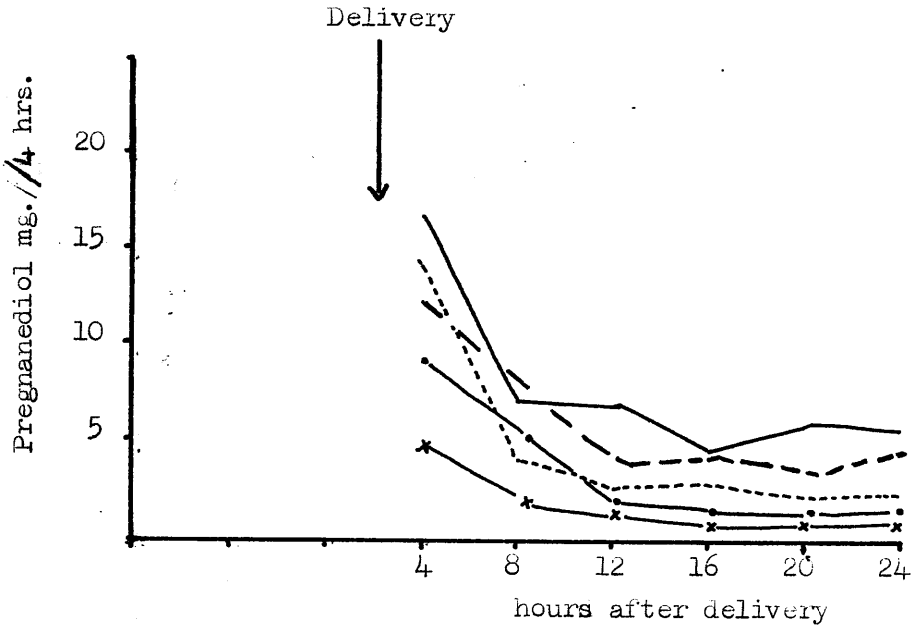


Fig. VII. THE 4 HOURLY PREGNANEDIOL EXCRETION IN 5 NORMAL PREGNANT WOMEN FOR 24 HOURS AFTER DELIVERY.

fall to levels of 2 to 4 mg./ 4 hours and in the last 12 hours the excretion remained fairly constant (Fig. VII).

DISCUSSION.

The range of pregnanediol excretion in normal pregnancy for the method of Klopper et al (1954) was studied by making regular estimations at regular "intervals" throughout the course of pregnancy in 24 intelligent women. The range is lower than that obtained, by other workers, using precipitation techniques, Guterman (1954), Davis & Fugo (1947) and Michie (1953). It is higher than that obtained by other chromatographic techniques, de Watteville (1951) and Trolle (1955). This is understandable as it has already been shown that the former techniques estimate an impure form of pregnanediol and in the latter technique pregnanediol is purified by leaching with cold solvents and this may remove some of the extracted pregnanediol (Klopper et al (1954)).

The pregnanediol excretion in pregnancy reaches a peak level at the 37th week and there is usually a fall in the test two or three weeks preceding delivery

(Fig. II); but the results obtained in the study of individual cases show that, of the 17 patients who were under observation until the onset of labour 13 had a fall in the excretion during the 36 - 38th week of pregnancy; excretion was maintained at a high level in the other 4 cases (Fig. IV).

When daily determinations were made in 9 cases the excretion level did not fall before delivery (Fig. V & VI). These results confirm the observations of Stover & Pratt (1939) and Hain (1941).

The pregnanediol excretion during the 24 hours following delivery was found to be high. All the patients were catheterised immediately after the placenta was expelled thus the pregnanediol excreted during that period may have been present in the blood at the time of delivery. In 5 of the 9 cases the excretion was less than 6 mg./24 hours lower than the level before the onset of labour. In case I (Fig. VI), although the patient was catheterised after completion of the third stage of labour the 4 hourly collection of urine was not organised for $5\frac{1}{2}$ hours during which time the patient had passed

urine. This may account for the low value obtained in this case in the 24 hours after delivery (15 mg./24 hours). Bradshaw & Jessop (1953) obtained values ranging from 5 to 28 mg./24 hours during the first 24 hours after delivery and 1 - 2.7 mg./24 hours on the 3rd day. Trolle (1955) found that the excretion during the first 24 hours in 4 cases was the same as that before delivery (15 - 30 mg./24 hours) but he obtained a fall to nil in the course of the next 3 - 6 days.

A gradual fall in excretion was obtained during the puerperium by the method of Klopper et al (1954), levels of 2 - 4 mg. being found on the 10th day after parturition. When four hourly excretion rates were studied, excretion immediately after delivery was high (5 - 17 mg./24 hours) and then there was a gradual drop, the level being maintained for the 12 to 24 hours after delivery (Fig. VII

SUMMARY AND CONCLUSIONS.

The range of pregnanediol excretion throughout normal pregnancy was studied using the method of Klopper et al (1954). The range is wide. It varies

from 2 - 15 mg./24 hours at the 10th week to 27 - 68 mg./24 hours at the 37th week. In most cases there is a fall in excretion in the weeks preceeding labour but in 4 of the 17 patients studied here the excretion was maintained.

When daily determinations were made for 10 days preceeding the onset of labour little variation was found in 3 patients but one patient had a 10 mg./24 hours rise in excretion.

After delivery the pregnanediol excretion was maintained at a high level for 24 hours and then there was a gradual fall to a level of 2 - 4 mg./24 hours on the 10th day after parturition. More than $\frac{1}{3}$ of the pregnanediol excreted during the 24 hours after delivery appears in the urine in the first 4 hours.

CONCLUSION

The pregnanediol excretion in normal pregnancy for the method of Klopper et al (1954) is now established. There is no specific variation characteristic of the approach of labour in the daily excretion but in the majority of cases the excretion

reaches a peak level at about the 36 to 38th week of pregnancy and then there is a gradual fall before delivery.

During the first 24 hours after delivery the excretion is only slightly lower than the excretion before delivery, the major part of this excretion occuring in the first four hours after delivery.

SECTION III.

THE PREGNANEDIOL EXCRETION IN THREATENED AND IN RECURRENT ABORTION, AND IN HYDATIDIFORM MOLE.

Among the many factors which are alleged to play a part in the aetiology of spontaneous abortion hormonal imbalance occupies an important place; there is as yet little knowledge and few measures of what constitutes hormonal balance. This section is concerned with only one aspect of the problem, the excretion of pregnanediol. Opinion as to the value of this assay differ; there are some who have found the pregnanediol excretion to be a reliable guide by demonstrating subnormal values preceding abortion (Venning 1937, Cope 1940, Guterman 1947, Bishop 1948, Guterman & Tulsky 1949, & Borglin 1956); whereas others maintain that normal values do not exclude the possibility of impending abortion, and doubt the significance of low values, (Hamblen, Cuyler & Baptist 1942, Swyer 1949, Plotz & Darup 1950, Zander 1951 and Swyer & Daly 1953).

Despite these uncertainties, progesterone has been used in the prevention of abortion (Bender 1948, Guterman 1953, Bishop, Richards & Doll 1950) and Smith & Smith (1948), considering that the metabolism of oestrogen and progesterone were interdependent, advised the administration of oestrogen and claimed an increase in the pregnanediol excretion when stilboestrol was administered during pregnancy. Similar findings were reported by Davis & Fugo (1947 and Sommerville, Marrian & Clayton (1949).

Guterman (1953) maintained that when progesterone was injected intramuscularly in cases of threatened abortion, abortion ensued if under 20% of the progesterone was converted into pregnanediol but if the conversion rate was about 20%, the pregnancy continued undisturbed.

Most workers who have assessed the progesterone metabolism in early abnormal pregnancy have used the methods for extracting and purifying pregnanediol which according to Marrian (1954) are not sufficiently sensitive, specific or accurate for the small amounts of pregnanediol found in early pregnancy urine.

The investigation detailed here is a reassessment of the value of the pregnanediol assay in the management of cases of threatened and recurrent abortion using the more reliable method of Klopper et al (1954). It is divided into 4 sections:-

1) The pregnanediol excretion in cases of threatened and recurrent abortion where no specific form of therapy was used;

2) The pregnanediol excretion in cases of recurrent abortion when stilboestrol therapy was used:

3) The conversion rate of exogenous progesterone in 15 cases of threatened abortion; and

4) The pregnanediol excretion in 5 cases of Hydatidiform mole.

THE PREGNANEDIOL EXCRETION IN THREATENED ABORTION.

Patients with a history of vaginal bleeding and abdominal pain in early pregnancy had a twenty four hour specimen of urine collected on admission to hospital. Cases in which abortion occurred within 3 days of admission were not included in the series, as they were assumed to have been inevitable or incomplete when first seen. In a series of 70 primary

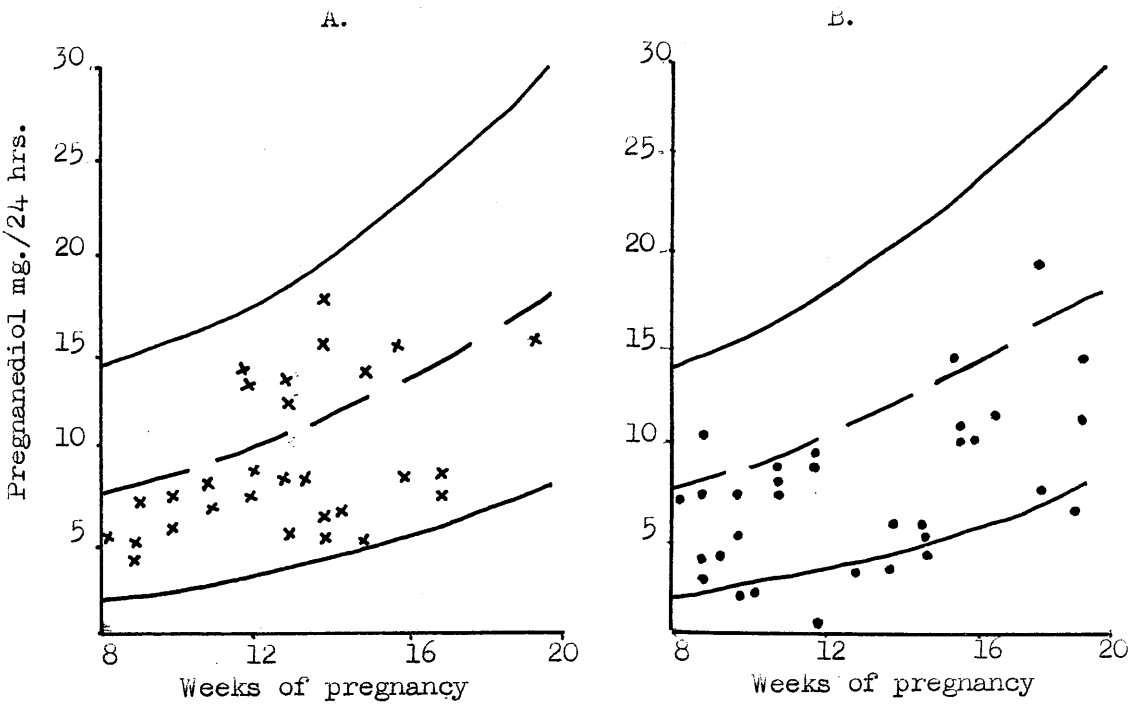


Fig. I. THE PREGNANEDIOL EXCRETION IN 61 CASES OF PRIMARY THREATENED ABORTION

A. ××× Live births (29 cases)

B. • • • Abortions (32 cases)

——— Normal pregnancy range.

— — Average readings for normal pregnancy.

threatened abortions, single pregnanediol estimations were made in 61 cases the remaining 9 cases having estimations carried out at weekly intervals. The duration of pregnancy at the time when the patients threatened to abort varied from 8 to 20 weeks. The results are shown on Fig. I. Of the 61 patients, 29 delivered live babies and 32 aborted. Before the 12th week of pregnancy there was no difference between the results obtained in the live birth and the aborted group. After that period 7 (38%) of the results obtained in the live birth group were above the average values obtained in normal pregnancy, whereas in the aborted group only 2 (10%) were above it; moreover in the latter group 5 (26%) of the readings were below the limits of the normal pregnancy range as compared with the live birth group where all the values were within the normal pregnancy range.

Pregnanediol estimations were made at weekly intervals in 9 cases; of these 5 delivered live babies (29 observations) and 4 aborted (17 observations). The results for each week of pregnancy are shown on Fig. IIa and IIb. which are scatter diagrams of all

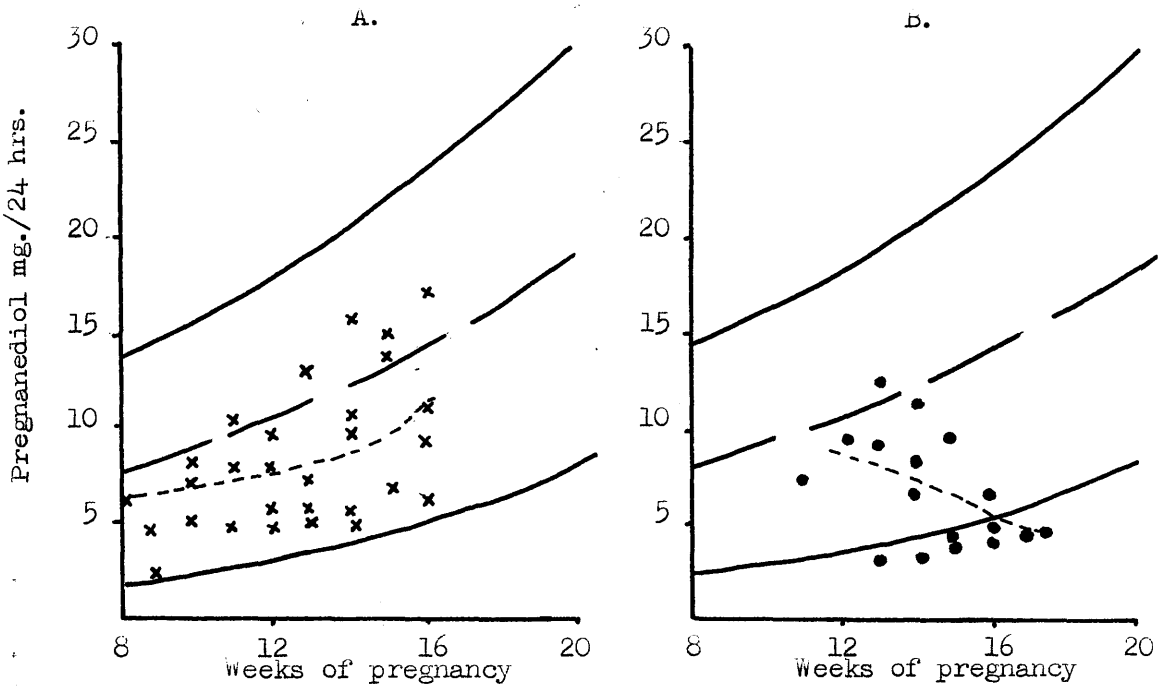


Fig. II. THE PREGNANEDIOL EXCRETION IN 9 CASES OF PRIMARY THREATENED ABORTION.

A. ××× Live Births (5 cases)

B. ●●● Abortions (4 cases)

——— Normal Pregnancy Range

— — Average readings for Normal Pregnancy

------- Average readings for cases of Threatened abortions.

Table I. THE PREGNANEDIOL EXCRETION IN 9 CASES OF PRIMARY THREATENED ABORTION.

Threatened abortion (live birth) 5 cases

Hospital No.	Weeks of pregnancy 8	9	10	11	12	13	14	15	16
8284	6.9	5.0	7.2	8.2	8.0	7.4	10.2	14.0	
10900					5.2	5.5	5.8	7.2	9.2
876				10.8	10.0	13.2	16.0		17.0
12558		2.4	5.6	5.0			5.3		6.2
36055			5.8		5.7	5.7	15.0	15.1	11.3
Average readings	6.9	3.7	7.2	7.7	7.2	8.9	10.5	12.1	10.9

Threatened abortion (aborted) 4 cases

Hospital No.	Weeks of pregnancy 8	9	10	11	12	13	14	15	16	17
13876							6.9	5.1	4.5	4.9
1402					9.8	12.6	11.5	9.2	6.5	
2243						3.3	3.7	4.4	5.2	4.9
9758				7.3	7.9	9.2	8.2			
Average readings				7.3	8.9	8.4	7.6	6.2	5.4	4.9

the readings obtained in these 2 groups of patients. The values obtained for the pregnanediol excretion are similar to those obtained when only single estimations were made. When, however the trend in individual cases is studied there is some evidence that declining results indicate that abortion is likely, and rising levels that the pregnancy will continue. These changes are more marked after the 12th week by which time careful clinical examination should give as much information as the hormonal assay. Nevertheless, the trend after the 12th week might on occasion be of value in adding weight to a clinical judgement.

THE PREGNANEDIOL EXCRETION IN RECURRENT ABORTION.

This group of patients received no specific form of treatment during this pregnancy. They had a history of 2 or more abortions in previous pregnancies. There were 13 patients in the series, 8 delivered live babies and 5 aborted. They were kept under observation throughout the course of pregnancy and urine was collected at daily or weekly intervals.

The results of the weekly pregnanediol estimations are shown on Fig. III a and b, which are scatter

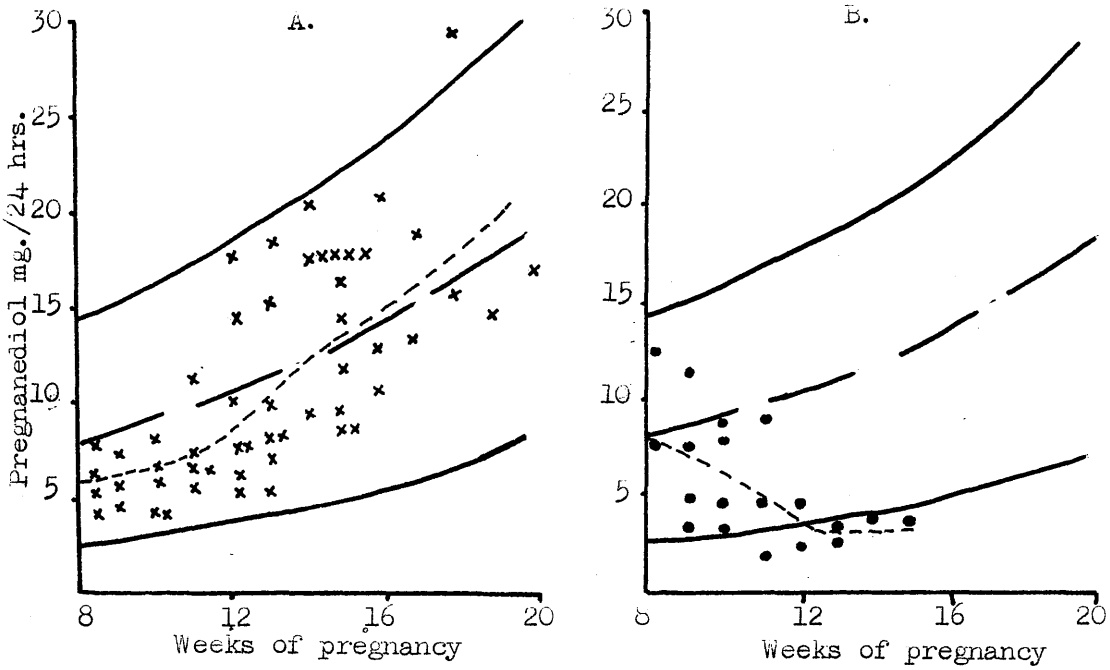


Fig. III. THE PREGNANEDIOL EXCRETION IN 13 CASES OF RECURRENT ABORTION WHEN NO SPECIFIC FORM OF THERAPY WAS USED IN THE NEXT PREGNANCY.

A. ××× Live births (8 cases)

B. ••• Abortions (5 cases)

——— Normal pregnancy range.

—— —— Average readings for normal pregnancy.

------ Average readings for recurrent abortions.

Table II a. THE PREGNANEDIOL EXCRETION IN 8 CASES OF RECURRENT ABORTION, WHO HAD NO SPECIFIC TREATMENT IN THE NEXT PREGNANCY AND WHO DELIVERED LIVE BABIES

Recurrent Abortions (live births)

Hospital No.	No of Previous Abortions	Weeks of Pregnancy												
		8	9	10	11	12	13	14	15	16	17	18	19	20
4755	2	5.7	5.5	6.4	11.2	14.2	15.7	17.4	17.4					
961	6			4.9	6.0	6.25	4.0	8.0	11.6	14.0				
6465	3					17.4	18.6	17.4	16.0	17.5	18.7	29.0		
6380	3	8.2	6.4	4.8	6.0	5.3	7.0		8.2					
45560	3	5.2	6.1	8.8	7.2	7.4	8.0	9.2	14.0					
23344	4	6.7	7.3	6.0	6.4	7.5	7.8	7.6	9.3	10.0				
27701	2					10.2	10.0	20.0	17.2	20.6				
727	3						5.5	6.8	8.3	12.7	13.1	15.5	14.2	16.9
Average readings		6.5	6.3	6.6	7.4	9.8	9.6	12.3	12.8	14.9	15.1	22.3	14.2	16.9

Table II b. THE PREGNANEDIOL EXCRETION IN 5 CASES OF RECURRENT ABORTION WHO HAD ANOTHER UNSUCCESSFUL PREGNANCY.

Recurrent abortions (aborted) 5 cases

Hospital No.	No. of Abortions	Weeks of pregnancy. 8	9	10	11	12	13	14	15
6081	3					4.1	3.0	4.0	3.5
1960	4		4.2	4.1	1.5	2.0			
428	4	12.4	11.3	8.8	4.5				
7758	2	8.0	7.4	8.2	9.0				
11956	3		3.2	3.0		2.0	2.5		
Average readings		8.2	6.3	6.0	4.7	2.6	2.3	4.0	3.5

diagrams of the results obtained in the 8 patients who delivered live babies and in the 5 who aborted. Table II shows the individual results for each patient and the average readings for each group. The results are similar to those obtained in the live birth groups and aborted group of threatened abortions. All the readings in the live birth group are within the normal pregnancy range and the average readings rise after the 12th week of pregnancy to above the normal pregnancy average. In the aborted group 7 results lie outside the lower limit of the normal range and the average readings show a fall after the 12th week of pregnancy.

The results in this group are similar to those in the group of primary abortions. Values below the normal range were associated with abortions, values below the average but still within the normal range did not appear to have any clear prognostic value, and values within the normal range but above the average were usually associated with continuing pregnancy.

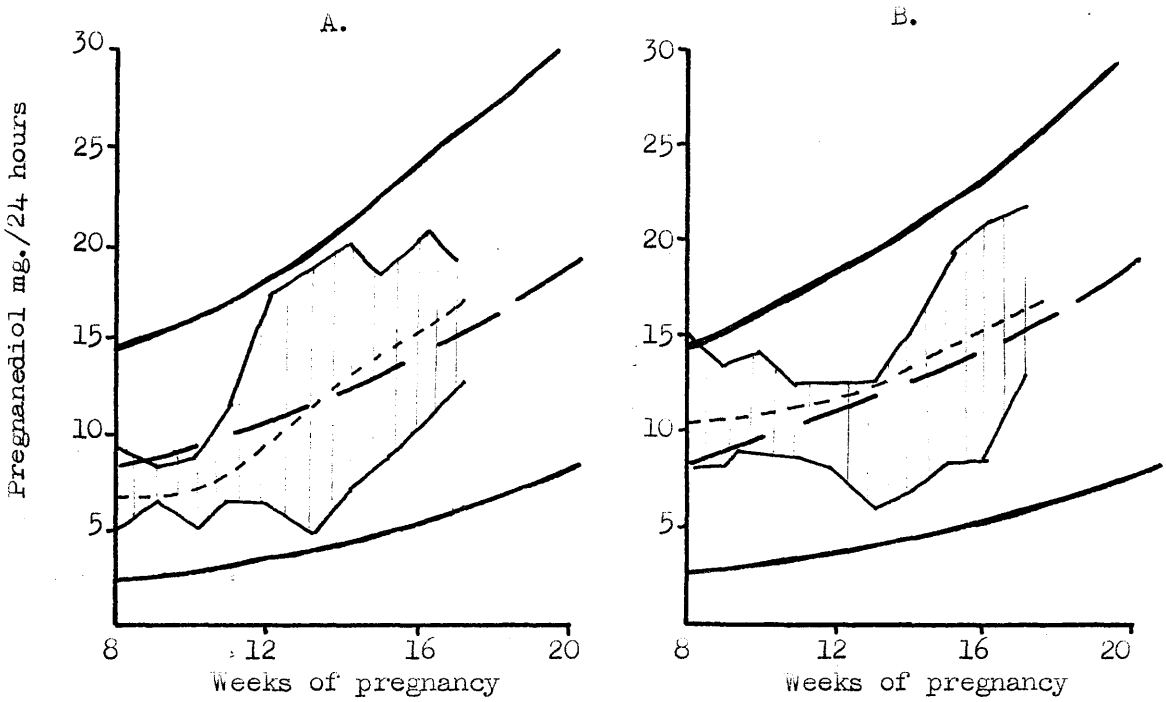


Fig. IV. THE PREGNANEDIOL EXCRETION IN CASES OF RECURRENT ABORTION WHO DELIVERED LIVE BABIES IN THE NEXT PREGNANCY.

A. No specific therapy (8 cases)

B. Stilboestrol therapy (6 cases)

The shaded areas show the limits of the lowest and highest readings obtained.

——— Normal Pregnancy Range

— — Average readings for normal pregnancy.

Table III a. THE PREGNANEDIOL EXCRETION IN 6 CASES OF RECURRENT ABORTION, TREATED WITH STILBOESTROL THERAPY, WHO DELIVERED LIVE BABIES.

Hospital Number	Number of abortions	Weeks of pregnancy 8	9	10	11	12	13	14	15	16	17	18	19
7704	2	8.8 ↓	8.6		8.4	8.8		14.8	15.0	16.8			
5732	2	15.2 ↓	13.0	14.2	12.4	8.0	8.8	12.4	16.4	21.0	22.0		
6106	3	9.5 ↓	10.4	11.4	11.8	12.6	12.0	15.7	19.2	20.6			
3573	3						5.5 ↓	6.2	8.2	8.8	14.2	15.0	
5080	3						6.4 ↓	7.7	9.3	9.5		10.4	12.0
63	3				12.0 ↓	12.4	12.4	13.0	14.0	15.0	16.2	17.4	17.2
Average		11.2	10.7	12.8	11.1	10.5	9.0	11.6	13.7	15.4	15.7	14.2	14.6

Table III b. THE PREGNANEDIOL EXCRETION IN ONE CASE OF RECURRENT ABORTION, TREATED WITH STILBOESTROL THERAPY, WHO HAD AN ABORTION.

Hospital Number	Number of abortions	Weeks of pregnancy 11	12	13	14	15	16	17	18	19	20	21	22	23	24
5149	2	9.8 ↓	10.5	10.2	11.4	10.3	16.3	19.1		25.2	17.5	16.7	18.0	17.5	15.8

↓ Indicates when the stilboestrol therapy commenced.

Table 4. THE DAILY PREGNANEDIOL EXCRETION IN 6 CASES OF RECURRENT ABORTION WHO DELIVERED LIVE BABIES

	No Specific Therapy			Stilboestrol Therapy		
Hospital Number	127	4755	961	6465	5080	65
Number of Abortions	3	2	6	3	3	3
Week of Pregnancy	13th	6th	10th	12th	12th	11th
Daily Pregnanediol Excretion	5.8 ←	1.5	5.7 ←	16.3	6.7 ←	12.8 ←
	5.9	2.6 ←	5.8	18.0 ←	6.5	11.2
	5.7	3.1	3.6	17.7	6.1	13.6
	5.0	3.0	3.5	15.7		12.0
		1.1	4.4	15.0	6.5	11.6
	5.0		3.5	18.8	5.8	11.6
		1.8	5.1	20.0	6.8	12.4
Week of Pregnancy	14th	7th	11th	13th	13th	12th
Daily Pregnanediol Excretion	6.8	2.4	4.7	19.9	6.8	13.0
		3.3	6.0	20.6		12.8
	6.9	4.5	7.8	19.4	8.0	12.0
		3.3	5.9	20.0		13.2
	6.6		4.5	15.9	7.2	
		2.9	6.5			12.0
	6.9	6.7	5.9	15.8	9.0	
Week of Pregnancy		8th	12th	14th	14th	13th
Daily Pregnanediol Excretion		5.7	6.5	16.0	10.6	11.6
		5.8		17.5		
			6.0	18.7		
		5.8				

← Indicates when the stilboestrol therapy was commenced

2) THE PREGNANEDIOL EXCRETION WHEN STILBOESTROL THERAPY WAS USED IN THE TREATMENT OF 7 PATIENTS WITH A HISTORY OF RECURRENT ABORTION.

Stilboestrol in increasing doses was administered to 7 patients with a history of 2 or more abortions in previous pregnancies. The initial dose was 30 mg. daily until the 16th week of pregnancy when it was increased by 5 mg. daily at weekly intervals until the 36th week. Six patients delivered living babies at or near term and one patient aborted at the 24th week of pregnancy.

The daily pregnanediol excretion in 2 patients who were treated with stilboestrol and in 4 of the untreated group are shown on Table IV. The daily excretion in both cases was constant and the stilboestrol treatment neither increased nor decreased the pregnanediol excretion. The results of the weekly determinations are shown on Table III where this is again demohstrated. In Fig. IV. a and b the mean values and the range of excretion are plotted against the mean range of normal pregnancy. The range in the recurrent abortion group is represented by the highest and lowest values obtained

for each week of pregnancy. The range in both groups lies between the normal pregnancy range and that obtained in the stilboestrol-treated group is neither higher nor lower than in the untreated group.

The pregnanediol excretion in the patient who aborted at the 24th week of pregnancy is shown on Table III. There was a rise till the 20th week when a slight fall occurred, the excretion rate being 15.5 mg./24 hours when the patient aborted.

3) THE CONVERSION OF EXOGENOUS PROGESTERONE IN THREATENED ABORTION.

Fifteen patients between the 9th and 20th week of pregnancy were admitted with a history of vaginal bleeding and abdominal pain and had pregnanediol assays made on 3 consecutive days after admission. On the 4th day 90 - 100 mg. of accurately weighed progesterone in 2 ml. sesame oil was injected intramuscularly. The percentage of the progesterone excreted as pregnanediol was then calculated from the pregnanediol excretion during the next 3 to 4 days.

The progesterone conversion in non-pregnant states

Table 5. THE PROGESTERONE RECOVERY IN 15 CASES OF THREATENED ABORTION.

Duration of Pregnancy	LIVE BIRTHS Recovery %	Pregnanediol Excretion before injection	Duration of Pregnancy	ABORTION Recovery %	Pregnanediol Excretion before injection
12 weeks	2.4	5.5	13	Nil	10.7
11	7.0	10.8	16	5.8	9.0
14	7.0	17.0	20	6.3	10.9
16	11.6	14.4	11	6.4	7.3
18	26.0	7.0	16	8.6	5.0
17	27.0	9.0	13	10.9	3.3
13	30.0	7.5	10	17	2.5
			9	29	4.0
Average	15%			10%	

was found by injecting the same amount in the proliferative and in the secretory phase of a menstrual cycle. The recovery was 9% in the proliferative phase and 11% in the secretory phase. These results agreed with those of Klopper & Mitchie (1955) and the experiment was not repeated.

In the threatened abortion group of 15 patients 8 aborted and 7 retained the pregnancy (Table V). The conversion rate in the aborted group varied from zero % to 29% and in the live birth group from 2.4% to 30%. The amount of progesterone converted bore no relationship to the urinary pregnanediol level nor the duration of pregnancy at the time of the injection. The conversion rate was not lower in the aborted group than in the other. Thus the progesterone conversion test was of no value in determining the outcome from pregnancy in this small series of cases.

4) THE URINARY PREGNANEDIOL EXCRETION IN 5 CASES OF HYDATIDIFORM MOLE.

These patients came under observation in the course of the investigation into the pregnanediol excretion in cases of threatened abortion. They were

Table 6. THE PREGNANEDIOL EXCRETION IN 5 CASES OF HYDATIDIFORM MOLE.

	Before operation	3 days after operation	7 days after operation	28 days after operation	6 weeks after operation	10 weeks after operation
1	26	23	17.0	12.8	3.2	0.7

	Before operation	1 day after operation	2 days after operation	7 days after operation	3 weeks after operation
2	6.6	2.8	2.8	2.0	1.5
3	8.8	7.8	3.4	3.2	1.6
4	16.2	10.8	7.8	0.75	
5	3.2	2.2	0.9	0.75	

all from 14 to 18 weeks pregnant and in all cases the Ascheim-Zondek test was positive in high dilution. The results of the pregnanediol excretion are shown on Table VI. Case I is the most interesting; she was 14 weeks pregnant and the size of the uterus corresponded to a pregnancy of 20 weeks. The Ascheim-Zondek test was positive in a 1 in 1000 dilution. The ovaries were enlarged to 4 times the normal size by large theca-lutein cysts. The pregnanediol excretion was 26 mg./24 hours before evacuation of the mole and 23 mg./24 hours the next day, 17 mg. 7 days later, 12.8 mg. after 28 days, 3.2 mg. after 6 weeks and 0.7 mg. after 10 weeks. The gradual wall in the pregnanediol excretion was roughly parallel to the reduction in the size of the ovaries which were palpably enlarged to twice normal size on vaginal examination 28 days after the operation, and were only slightly enlarged 4 weeks later.

In Case 4 the ovaries were enlarged to about 5 times normal size by theca-lutein cysts and the pregnanediol excretion was 17 mg./24 hours. The mole was removed by abdominal hysterectomy and about 75% of the ovaries, which were enlarged by theca-lutein cysts,

were removed at the same time. Thus the regression of the pregnanediol excretion was not so marked in this case.

In the other 3 cases no palpable enlargement of the ovaries was noted. The pregnanediol excretion before evacuation of the uterus varied from 8.8 - 2.4 mg./24 hours and after evacuation it gradually fell to normal levels.

DISCUSSION.

The pregnanediol excretion in 70 cases of primary threatened abortion was studied. Before the 12th week of pregnancy the results obtained did not give any indication of the final outcome of the pregnancy in the majority of the cases. After the 12th week significantly lower results were obtained in the group where abortion occurred. When the results obtained in the individual cases were studied it was found that in cases where excretion fell, the patient was more likely to abort than in cases where a normal level of excretion was maintained. However in 3 cases where abortion occurred the excretion level was maintained (Tables I and II).

The bleeding in threatened abortion is due to haemorrhage into the chorio-decidual space. This may upset the nutrition of the trophoblast and cause foetal death but the progesterone production of the trophoblast will only cease when the damage is sufficient to cause degeneration of large areas of placenta. The cases investigated here show that it is difficult to diagnose placental degeneration, before the 12th week of pregnancy, by the pregnanediol excretion because the range of excretion in normal pregnancy is so wide that it is difficult to detect abnormal variations. Moreover placental degeneration is not the only cause of abortion in early pregnancy. The foetus may die from some other cause, e.g. congenital defect and the placenta may still survive.

When the daily or the weekly pregnanediol excretion was determined in 6 patients treated with stilboestrol who had a history of abortions in previous pregnancies, neither an increase nor a fall in excretion was noted. The range of excretion compared favourably with that found in normal pregnancy (Fig. III). The dosage of stilboestrol used was similar to that

recommended by Smith & Smith (1948) who demonstrated that stilboestrol increased the pregnanediol excretion during pregnancy. Sommerville, Marrian & Kellar (1948) found that there was a fall in excretion during the administration of 50 mg. of stilboestrol orally. The findings in the small series of cases followed here confirm the work of Michie (1955) who could not demonstrate a rise nor a fall after the oral administration of stilboestrol.

The management of cases of recurrent abortion have long aroused controversy. In 8 patients who received no specific form of therapy and where the outcome of the pregnancy was successful, wide variation in the pregnanediol excretion, between patients, occurred. These were more marked after the 12th week of pregnancy (Fig. IIb).

Patients with low levels of excretion had as successful an outcome from pregnancy as those with high levels and Bevis (1951) and Swyer & Daly (1953) stressed the part played by psychotherapy in the management of these cases. King (1953) summarised the results obtained by 14 authors who used a variety of treatments

and came to the conclusion that the success rate was not altered by hormone replacement therapy.

When large doses of progesterone (90 to 100 mg.) were injected into patients threatening to abort, the amount recovered did not depend onthe outcome of the pregnancy. Venning & Browne (1940) and Sommerville & Marrian (1950) observed that there was a higher conversion of progesterone to pregnanediol in pregnancy than in the non-pregnant state. Guterman (1953) showed that in cases of threatened abortion the conversion was lowere where abortion occurred than were the pregnancy continued. Borth (1954) confirm this but Robson & Gornall (1951) found this test to be of little value. Klopper & Michie (1955) obtained a conversion rate of about 16% in men, pregnant and non-pregnant women when a single injection of 50 mg. of progesterone was given. The conversion rate in the present series was 15% in the live birth group and 10% in the group which aborted. The range of values obtained was 2.4% to 30% in the latter group and zero % to 29% in the former. Thus the conversion rate in this series varied considerably; it was not influenced

by the outcome of the pregnancy, the initial pregnanediol excretion nor the duration of pregnancy.

The high level of pregnanediol excretion over a six weekly period after evacuation of a hydatidiform mole deserves special mention. The large theca-lutein cysts of the ovaries must have been responsible for the production of progesterone in this case. In the other cases where the ovaries were not enlarged the pregnanediol excretion before evacuation of the uterus varied from 2.8 to 7.8 mg./24 hours and there was a rapid return to normal levels after evacuation. Plotz & Darup (1950) reported the continued excretion of pregnanediol in large quantities for 20 days after evacuation of a hydatidiform mole, but they did not associate it with theca-lutein cysts of the ovaries.

SUMMARY.

This investigation was undertaken to assess the value of the urinary pregnanediol assay in the management of cases of threatened and recurrent abortion. The chromatographic method of Klopper et al (1954) was used in the extraction and purification of the pregnanediol.

The assay was found to be of very little value in determining the outcome of pregnancy in cases of threatened abortion before the 12th week of pregnancy. After the 12th week a lower level of excretion was found in the cases where abortion occurred than in the cases where the pregnancy continued.

In 8 patients with a history of recurrent abortions in previous pregnancies who received no specific form of therapy in the next pregnancy and who delivered live babies, the pregnanediol excretion varied from 5 to 15 mg./24 hours at the 12th week of pregnancy. The range of excretion was within the normal pregnancy range.

When stilboestrol was used in the treatment of 7 patients with a history of recurrent abortion the range of the pregnanediol excretion was again within the normal pregnancy range and neither an increase nor a decrease in the pregnanediol excretion was noted. One of the patients aborted at the 24th week of pregnancy and a falling excretion was noted before abortion occurred.

When one injection of (90 - 100 mg.) progesterone was given to 15 patients with a history of threatened abortion the amount recovered as pregnanediol was not related to the outcome of the pregnancy, the duration of pregnancy or the initial pregnanediol excretion.

In one case of hydatidiform mole where large theca-lutein cysts of the ovaries were present the pregnanediol excretion was abnormally high (26 mg./ 24 hours) for the duration of pregnancy and it remained high after evacuation of the mole, returning to normal limits when the ovarian enlargement had subsided 6 weeks later. In 3 cases where there was no ovarian enlargement the pregnanediol excretion was lower and normal values were found 2 to 4 weeks after evacuation of the moles.

CONCLUSION.

It may be concluded that the estimation of the urinary pregnanediol excretion in the early months of abnormal pregnancy is seldom helpful because in normal pregnancy the excretion varies over such wide limits. There is a trend however which suggests that after the 12th week of pregnancy an excretion level below the normal average for the period of gestation

carries a somewhat worse prognosis than one above the normal average.

In the small series of cases of recurrent abortion, detailed here, hormone replacement therapy with stilboestrol did not appear to increase or decrease the pregnanediol excretion.

The conversion rate of progesterone to pregnanediol in 15 cases of threatened abortion was not influenced by the duration of pregnancy, the initial pregnanediol excretion nor the outcome of the pregnancy.

An abnormally high level of excretion in early pregnancy may indicate the presence of a hydatidiform mole and theca-lutein cysts of the ovaries.

SECTION IV.

THE PREGNANEDIOL EXCRETION IN TOXAEMIA OF PREGNANCY AND ESSENTIAL HYPERTENSION.

INTRODUCTION.

The stillbirth and neonatal mortality from toxaemia of pregnancy ranges from 5% to 30% and it is higher in the severe than in the mild and moderate cases (Dieckman 1952). Induction of labour or Caesarean section, often performed before term in the severe cases of toxaemia, for the benefit of the mother results in the birth of premature infants. According to Cross (1952) 18% of the premature babies which die are delivered by toxaemic mothers. It is likely that the death of some of these babies is due to placental insufficiency causing malnutrition in utero.

The pregnanediol excretion has been used as an idex of placental efficiency, and according to de Watteville (1951) and Richli (1953) the foetal prognosis is poor if the pregnanediol levels are low. On the other hand Trolle (1955) showed that a low pregnanediol excretion was not always indicative of poor foetal prognosis. Other workers were unable to establish a

relationship between low pregnanediol excretion and the severity of the toxaemia (Venning, Henry and Browne, 1938; Hain, 1940 and Chandhuri et al 1953).

This investigation was undertaken to establish the value of the pregnanediol assay in the management of cases of toxaemia and essential hypertension. Regular pregnanediol estimations were performed on a number of these cases and they were kept under observation until delivery, when the type of delivery and the duration and character of the labour were noted. The weight and condition of the baby and the weight, macroscopic and histological appearance of the placenta were also recorded.

Patients who came under observation during this investigation were referred for pregnanediol assays by the hospital staff on account of mild, moderate or severe toxaemic signs, or essential hypertension in early pregnancy. In some cases the investigation was undertaken because the uterine growth was less than was expected for the duration of ammenorrhoea, although the signs and symptons of toxaemia were very mild and improved after admission

to hospital for treatment.

The cases followed during the course of this investigation have been subdivided into:-

A. Toxaemia of pregnancy.

B. Essential Hypertension in pregnancy.

C. Stillbirths and Neonatal deaths associated with toxaemia or Essential Hypertension.

D. Pregnancy associated with chronic renal disease.

RESULTS.

A. THE PREGNANEDIOL EXCRETION IN TOXAEMIA OF PREGNANCY.

The cases in this group all had living babies which survived. A subdivision is made into:-

1) The less severe cases which responded to treatment and were not delivered until after the 37th week of pregnancy.

2) The severe and fulminating cases which did not respond to treatment and which were all delivered between the 34th and 36th week of pregnancy, except one case which was delivered at the 38th week by Caesarean section.

Table 1 THE CLINICAL DETAILS OF 18 PATIENTS WHO HAD SEVERE TOXAEMIA AND WHO DELIVERED LIVE BABIES

Hospital Number	Case No.	Birth Weight	Placental weight	Highest Blood Pressure mm.Hg.	Albumen g/1000 ml.	Oedema	Duration of Toxaemia (weeks)	Duration of pregnancy at delivery (weeks)	Type of Delivery
6453	1	5lb. 1oz.	1 lb. 2oz	150/90	Trace	+	3	40	S.I. Spontaneous
4326	2	5lb. 7oz.	1 lb. 2oz.	140/86	+	+	4	38	Spontaneous
13877	3	5lb.14oz.	1 lb. 4oz.	160/100	2	+++	4	39	S.I. Spontaneous
8797	4	6lb.10oz.	1 lb. 6oz.	150/100	+	-	2	39	"
13532	5	5lb. 9oz.	1 lb. 2oz.	155/100	+++	+	4	39	"
10905	6	6lb.13oz.	1 lb.10oz.	150/100	-	++	5	37	Caesarean Section
48548	7	7lb.10oz.	1 lb.10oz.	150/90	+	-	4	40	Spontaneous
32104	8	5lb. 4oz.	1 lb. 4oz.	140/90	+	-	2	38	S.I. Spontaneous
9937	9	6lb. 4oz.	1 lb. 3oz.	150/100	+	+	-	40	Spontaneous
9613	10	6lb. 3oz.	1 lb.	150/110	+	-	2	40	S.I. Spontaneous
7928	11	(5lb. 7oz. (6lb. 9oz.	2lb. 2oz.	140/90	+	+	3	39	Spontaneous
7093	12	(5lb.10oz. (5lb.15oz.	2 lb.	150/90	0.5	+	4	38	"
44845	13	5lb.13oz.	1 lb. 4oz.	140/85	+	+	6	40	S.I. Spontaneous
11880	14	6lb. 2oz.	1 lb. 3oz.	160/100	++	-	4	37	"
8254	15	6lb.11oz.	1 lb.	150/100	++	+	6	38	"
7238	16	6lb. 3oz.	1 lb. 6oz.	150/90	1.0	+	4	39	Caesaream Section
10434	17	5lb. 3oz.	1 lb. 3oz.	160/100	2.5	+	7	38	S.I. Spontaneous
1240	18	6lb.10oz.	1 lb. 4oz.	140/95	1.0	+	4	38	"
Average		6lb. 1oz.	1 lb. 4oz.					38½	S.I. = Surgical Induction

1). The less severe cases of toxaemia.

In this group of 18 cases, all were delivered of live babies which survived; there were 2 cases of twin pregnancy. The duration of toxaemia varied from 3 to 7 weeks before delivery and after admission to hospital with the institution of treatment (rest and sedatives) there was a clinical improvement in all the cases, and the pregnancies were continued until after the 37th week. The clinical findings are shown on Table 1, the highest blood pressure recorded was 160/100 mm.Hg. and the albuminuria varied from a trace to 2.5 g. per 1000 ml. but in one case albumen was not present. In 5 cases oedema was not detected.

Living babies were delivered by Caesarean section in two cases, and vaginally in sixteen cases. Surgical induction by artificial rupture of the membranes was performed in 11 cases. Foetal distress was not detected in any case. The average weight of the babies was 6lb. 1oz. with a range of 5lb.1oz. to 7lb. 10oz. The average weight of the placentae was 1 lb. 4oz. with a range of 1 lb. to 1 lb. 10oz.

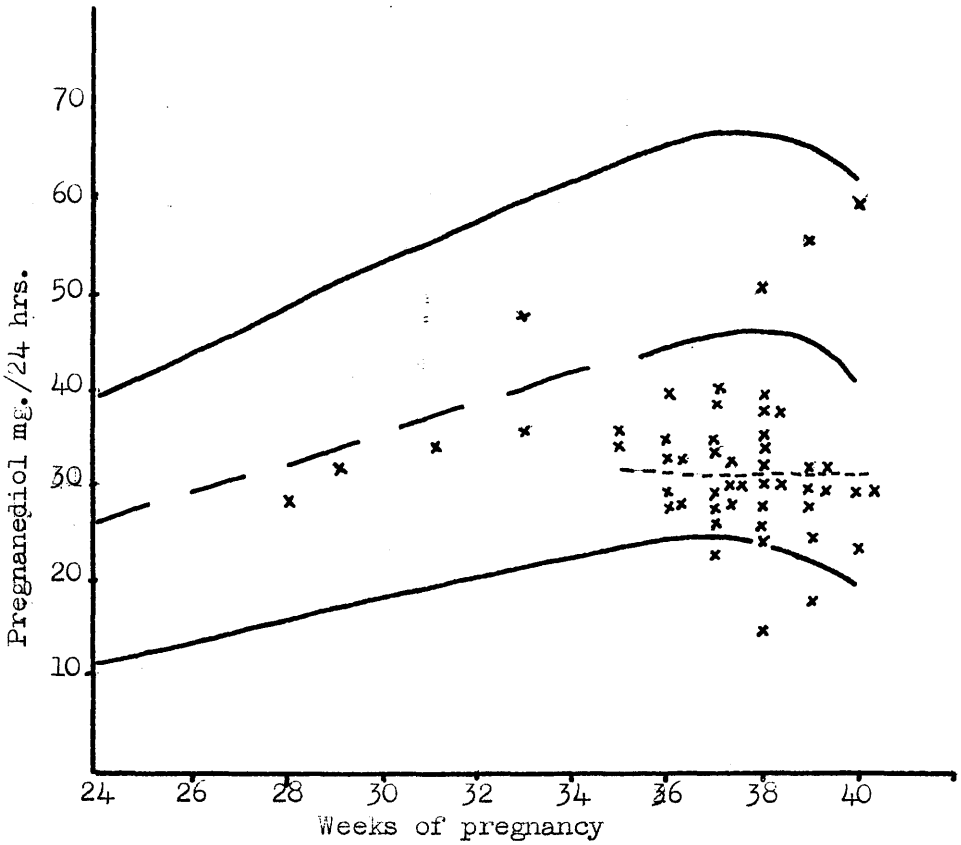


Fig. 1. A SCATTER DIAGRAM OF THE PREGNANEDIOL EXCRETION LEVELS, IN 18 CASES OF LESS SEVERE TOXAEMIA, WHO DELIVERED LIVE BABIES (Table 2)

——— Normal pregnaney range.

——— ——— Average readings for normal pregnancy.

- - - - - Average readings for Toxaemia Group.

Table 2. THE PREGNANEDIOL EXCRETION IN 18 CASES OF LESS SEVERE TOXAEMIA WHO DELIVERED LIVE BABIES

Case Number	Weeks of pregnancy												
	28	29	30	31	32	33	34	35	36	37	38	39	40
1													30.4
2											26.0		
3												28.4	
4									29.1	34.6	38.2	30.4	
5												17.5	
6						36.3		35.0	39.5	28.0			
7											50.4	56.0	60.4
8										29.6	30.4		
9										40.0	37.5	25.5	
10											24.0	28.0	24.0
11									28.1	32.4	30.8	32.4	
12										29.9	38.0		
13										38.8		32.2	30.0
14									32.5	29.1			
15									28.9	26.8	27.5		
16									35.6	35.0	35.2		
17	28.0	32.0		34.0		48.0		36.0	33.0	23.0	15.0		
18										28.0	34.0		
Average readings									32.4	31.3	32.2	31.4	31.2
Normal pregnancy average readings									46.0	57.7	42.4	45.6	41.9

the twin pregnancies being excluded. Thus both the babies and placentae in this group were of average for an average duration of pregnancy of 38½ weeks except 4 which were under 5½ lb.

The pregnanediol excretion (Fig.1, Table 2) was on the average low for the period of pregnancy. Case 5 showed a subnormal excretion (17.5 mg. at the 39th week) with the low baby weight of 5lb. 9oz. at 39 weeks' maturity. Case 10 showed a subnormal excretion at the 38th week but a slight rise at the 39th week brought it into the normal range. Case 17 showed a fall in excretion after the 33rd week, but it was not until the 37th and 38th weeks that the excretion became subnormal, surgical induction at the 38th week resulted in the spontaneous delivery of a live infant which weighed only 5lb. 3oz. Thus, of the 3 patients who showed subnormal excretion, only two were delivered of babies whose weight was low for the relevant gestation period. In cases 1, 2 and 8 unduly small babies for the respective gestation period were also delivered, the pregnanediol excretion was within the normal range, but below the normal average.

Table 3. THE CLINICAL DETAILS OF 9 PATIENTS WHO HAD SEVERE TOXAEMIA AND WHO DELIVERED LIVE BABIES.

Hospital Number	Case No.	Birth Weight	Placental Weight	Highest Blood Pressure mm/Hg.	Albumen g/1000 ml.	Oedema	Duration of: Toxaemia (weeks)	Labour (hours)	Pregnancy at delivery	Type of Delivery
54036	1	4lb. 8oz.	14oz.	145/95	3.0	++	2	8	36	S.I. Spontaneous
12397	2	2lb. 12oz.	13oz.	150/100	1.0	+++	7	-	35	Caesarean section
3818	3	4lb. 5oz.	1 lb.	180/100	8.5	+	6	8	34	S.I. Spontaneous
12218	4	3lb. 5oz.	1 lb.	140/90	2.0	+	5		36	Caesarean section
8170	5	4lb. 15oz.	1lb. 1oz.	150/100	6.0	++	2	7	35	S.I. Spontaneous
7721	6	4lb. 13oz.	1lb. 1oz.	150/110	2.0	+	6	-	38	Caesarean section
13125	7	3lb. 8oz.	14oz.	160/100	0.5	+++	5	16	35	Spontaneous
13886	8	4lb. 6oz.	13oz.	150/100	2.0	+++	10	-	36	Caesarean section
6673	9	4lb. 5oz.	13oz.	190/100	3.0	+	10	-	34	Caesarean section
Average		3lb. 10oz.	14oz.						36	

S.I. = Surgical Induction.

Considering all the 18 cases of toxaemia in which the pregnancy was terminated after the 37th week, the babies were all over 5 lb. in weight, and the placental weight over one pound. The average pregnanediol excretion, although within the range for normal pregnancy was below the mean excretion for the relevant duration of pregnancy.

2). Cases of severe and fulminating toxaemia.

Of nine patients considered in this group, some improved slightly with bed rest and sedatives, but none completely recovered, and the pregnancy was terminated between the 34th and the 36th week in 8 cases and at the 38th week in the remaining cases. The clinical details are shown on Table 3. The blood pressure range was from 140/90 to 190/100 mm.Hg. and the albuminuria from 0.5 g./1000 ml. to 8.5 g./1000 ml., all the patients had oedema. Five cases were delivered by caesarean section, 3 vaginally after artificial rupture of the membranes, and one spontaneously. They all had living babies which survived. The average weight of the babies was 3lb. 10oz., the range being 2lb. 12oz. to 4lb. 15oz. There was thus

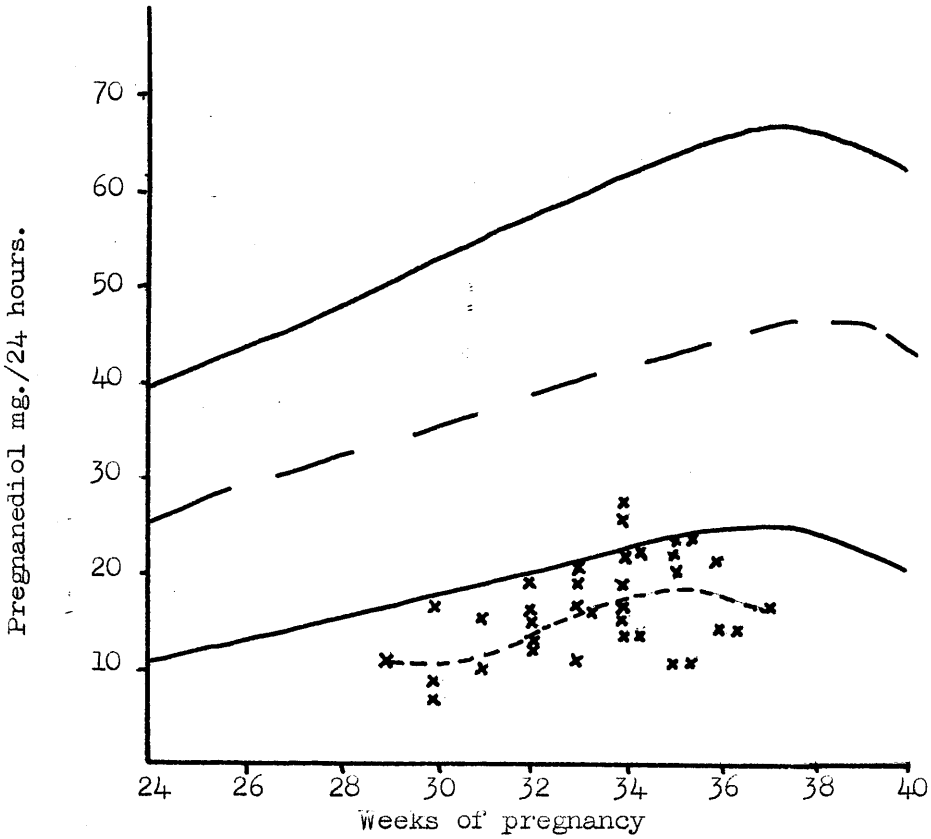


Fig. 2. .A SCATTER DIAGRAM OF THE PREGNANEDIOL EXCRETION LEVELS, IN 9 CASES OF SEVERE TOXAEMIA, WHO DELIVERED LIVE BABIES.

——— Normal pregnancy range.

— — Average readings for normal pregnancy.

— - - - - Average readings for the Toxaemia Group.

Table 4. THE PREGNANEDIOL EXCRETION IN 9 CASES OF SEVERE TOXAEMIA WHO DELIVERED LIVE BABIES

Case No.	Weeks of pregnancy. 29	30	31	32	33	34	35	36	37
1						22.0	24.0		
2		7.0	12.5	17.3	11.2	14.2	10.2		
3				18.5	20.4	26.1			
4						27.2	20.5	21.4	
5						18.1	20.0		
6	12.0			14.0		16.0	14.5	14.0	14.5
7		16.9		12.3	15.8	11.9			
8	10.4	8.1	10.0	17.8	19.1	21.7	24.0	13.5	
9					16.0	12.8			
Average Readings	11.2	10.7	11.3	16.0	16.5	18.9	18.9	16.3	16.5
Normal pregnancy Average Readings	36.4	37.2	26.9	37.8	39.3	40.7	45.4	46.0	57.7

a low average birth weight for an average duration of pregnancy of 36 weeks. One of the patients (Case 6) who was delivered by Caesarean section at the 38th week, had a 4lb. 13oz. baby which was asphyxiated at birth and had convulsions with cyanotic attacks for 2 to 3 days after delivery; it responded to treatment and survived.

The average weight of the placenta for this group was 15oz. ranging from 13 to 16oz. The pregnanediol excretion is shown on Fig. 2, and Table 4. It will be noted that the excretion in all but 2 cases was below the lower limit of the normal pregnancy range and that the average excretion was well below that range. The excretion levels for cases 2, 7 and 8 are shown in Figs. 3b and 4a and 4b., a fall in excretion was noted before delivery in each of these cases. The average excretion of pregnanediol in normal pregnancy falls at the 37th week, but in this group of cases the fall occurred between the 31st and 35th week and was associated with subnormal birth weights.

The pregnanediol excretion in case 6 is shown in Fig. 3a. It will be noted that a low level was

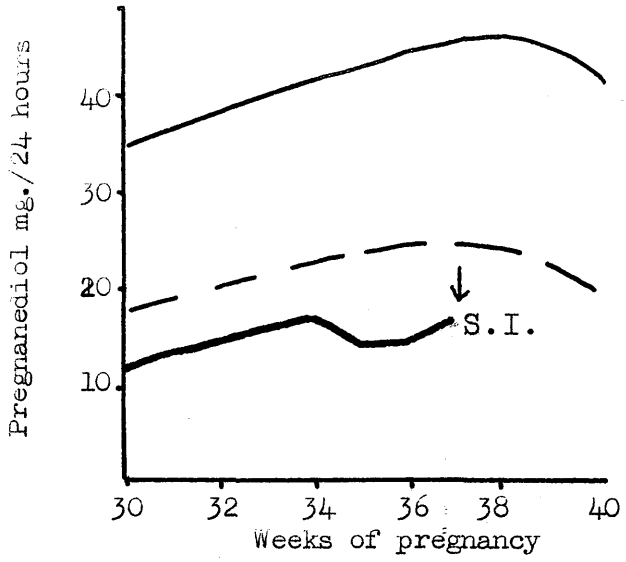
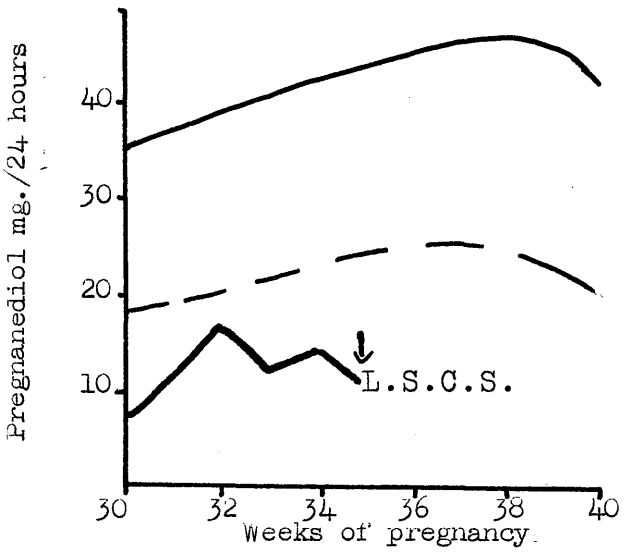


Fig. 3 a.

Case 6. (Table 4).

Baby 4lb. 13oz.(alive)

S.I. Surgical Induction
37 weeks.



Case 2. (Table 4)

Baby 2lb. 12oz. (alive)

L.S.C.S. Lower Segment
Caesarean Section
35 weeks.

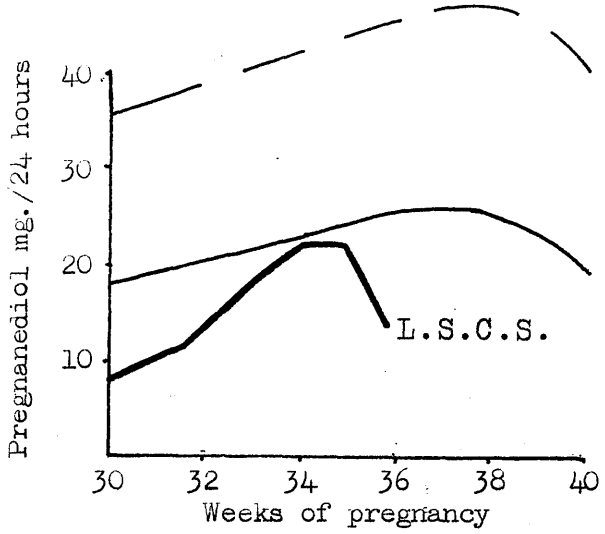
Fig. 3 b.

The pregnanediol excretion in 2 cases of severe Toxaemia.

——— Pregnanediol excretion.

— — Normal pregnancy range (lower limit)

——— Average readings for normal pregnancy.

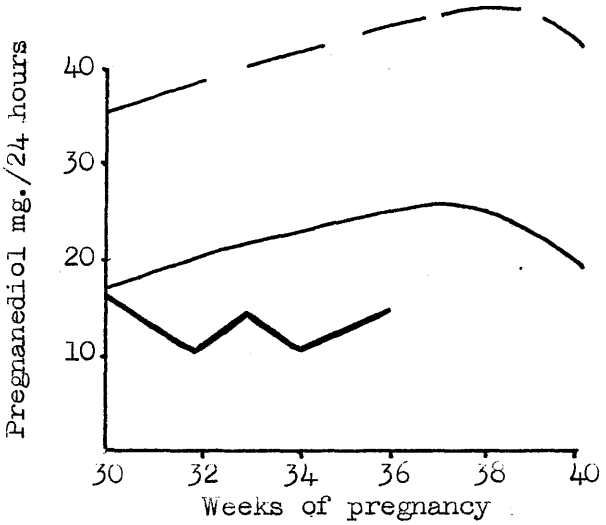


Case 8 (Table 4)

Baby 4lb. 6oz.(alive)

L.S.C.S. (Caesarean Section) 36 weeks.

Fig. 4a.



Case 7 (Table 4).

Baby 3lb. 8oz.(alive).

Spontaneous Delivery (35 weeks).

Fig. 4b. THE PREGNANEDIOL EXCRETION IN 2 CASES OF SEVERE TOXAEMIA.

——— Pregnanediol excretion.

——— Normal pregnancy range.

— — Average readings for normal pregnancy.

maintained till delivery at the 38th week. This is in agreement with the small size of the baby (4lb.13oz.)

Thus in this group there is good correlation between baby weight and pregnanediol excretion. An association between pregnanediol excretion and foetal prognosis cannot be posulated since all these babies survived.

Discussion.

In the two groups of toxaemia presented here, the only clinical difference was that the cases in the less severe group responded to rest and those in the severe group did not. The difference in pregnanediol excretion between the 2 groups was marked; the excretion was almost entirely subnormal in the severe group and almost entirely normal in the less severe group. The conclusion to be drawn is that subnormal pregnanediol values are associated with the types of toxaemia which are resistant to treatment and consequently low pregnanediol levels may indicate that the toxaemia is of this severe type. Live babies resulted from all these cases, so it cannot be concluded that low pregnanediol excretion is always associated with poor foetal prognosis; this

Table 5. THE CLINICAL DETAILS OF 13 PATIENTS WHO HAD ESSENTIAL HYPERTENSION NOT COMPLICATED BY TOXAEMIA.

Hospital Case	Case No.	Birth Weight	Placental Weight	Highest Blood Pressure mm/hg.	Duration of pregnancy at delivery	Type of Delivery
8918	1	4lb. 8oz.	1 lb. 1oz.	160/110	36	Caesarean section
1954	2	7lb. 11oz.	1 lb. 6oz.	140/90	40	Spontaneous
1428	3	7lb. 8oz.	1 lb. 12oz.	170/90	40	"
60013	4	5lb. 8oz.	1 lb. 1oz.	180/110	37	S.I. Spontaneous
7567	5	6lb. 2oz.	1 lb. 9oz.	175/110	41	Spontaneous
9365	6	6lb. 1oz.	1 lb. 6oz.	160/110	40	"
10890	7	7lb. 8oz.	1 lb. 1oz.	150/90	38	S.I. Spontaneous
8927	8	8lb. 1oz.	1 lb. 8oz.	180/100	40	Spontaneous
4422	9	(6lb. 7oz.) (5lb. 9oz.)	2 lb. 1oz.	150/90	40	"
14440	10	4lb. 1oz.	14oz.	160/110	38	Caesarean section
2498	11	6lb. 4oz.	1 lb. 1oz.	150/90	39	Spontaneous
11417	12	6lb.	1 lb. 3oz.	190/110	38	Forceps (Foetal distress. Labour 4 hours).
12194	13	7lb.	1 lb. 3oz.	160/90	40	Spontaneous
Average		6lb. 6oz.	1 lb. 4oz.			S.I. = Surgical Induction.

conclusion is in agreement with that of Trolle (1955) but in disagreement with that of de Watteville (1951) and Richli (1953).

B. THE PREGNANEDIOL EXCRETION IN PREGNANCY COMPLICATED BY ESSENTIAL HYPERTENSION.

The patients in this group were all delivered of live babies which survived. A subdivision of cases is made into:-

1) Essential hypertension not complicated by toxaemia.
2) Essential hypertension complicated by toxaemia.

1). Essential hypertension not complicated by toxaemia.

Table 5 shows the clinical details of 12 patients with single pregnancies and one with twin pregnancy. Caesarean section was performed at the 36th week in 2 cases and the remainder were delivered vaginally between the 37th and 41st week. In one case (Case 12, Table 5) foetal distress was noted after a 13 hour labour, but forceps delivery resulted in the birth of an asphyxiated baby which responded to treatment and survived.

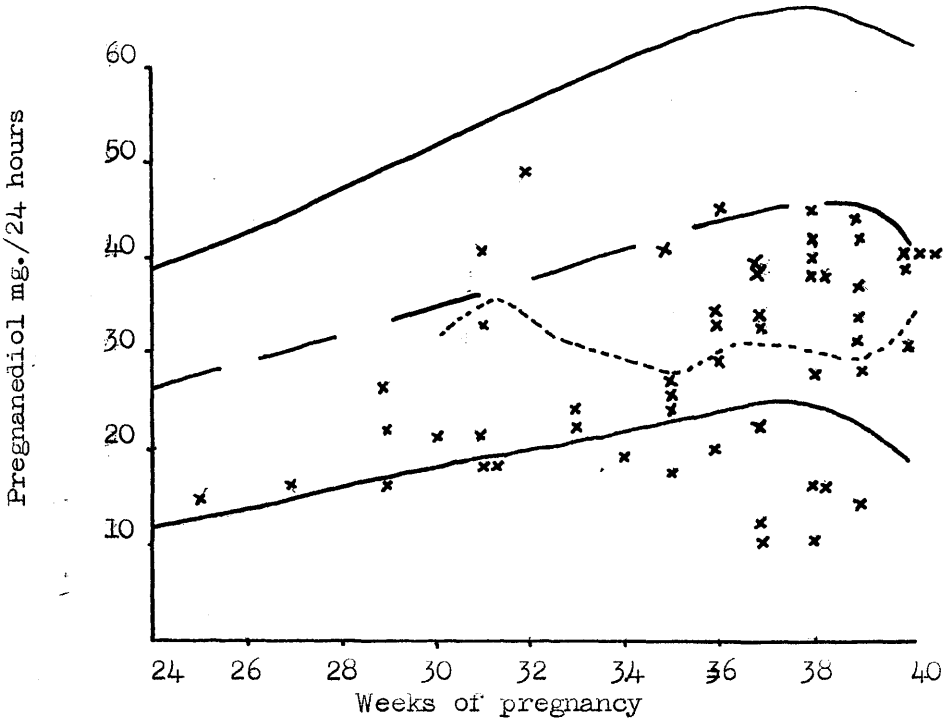


Fig. 5. A SCATTER DIAGRAM OF THE PREGNANEDIOL EXCRETION LEVELS, IN 13 CASES OF HYPERTENSION WITHOUT TOXAEMIA, WHO DELIVERED LIVE BABIES.

——— Normal pregnancy range.

—— —— Average readings for normal pregnancy.

-------- Average readings for hypertensive group.

Table 6. THE PREGNANEDIOL EXCRETION IN 13 CASES, OF ESSENTIAL HYPERTENSION NOT COMPLICATED BY TOXAEMIA, WHO DELIVERED LIVE BABIES.

Case Number	Weeks of pregnancy											
	29	30	31	32	33	34	35	36	37	38	39	40
1	16.4	21.0	20.5	22.4	23.6							
2			33.6	40.6						29.2		
3		42.8	41.2		42.0		40.0	44.5	39.0	38.0	42.0	40.0
4								18.5	22.0			
5								34.4	38.0	38.0	34.0	31.0
6										28.0	28.0	26.0
7									34.0	40.8		
8						18.8	25.0	33.0	33.0	45.6	44.2	40.2
9											37.0	39.2
10									10.0	10.2		
11	26.0		18.2		23.8		26.6		31.4	16.0	14.3	
12							17.0	19.6	11.4	16.4		
13											31.4	39.8
Average Readings			28.4		29.6		27.2	30.0	31.2	30.2	28.7	34.4
Normal pregnancy average readings			36.9	37.9	39.3	40.7	45.4	46.0	57.7	42.3	45.6	41.9

The lowest birth weights were found in the 2 cases delivered by Caesarean section, 4lb. 1oz., and 4lb. 8oz. The other babies weighed more than 5lb. 8oz. The placenta of the 4lb. 1oz. baby weighed 14 oz. and the others weighed over 1 lb. Thus both the birth and the placental weights corresponded with the appropriate gestation period except in the 2 cases delivered by Caesarean section. In one of these the baby and the placental weight was low and in the other the baby weight was low.

The pregnanediol excretion is shown in Fig. 5 and Table 6. It can be seen that although some of the results lie below the level limit of the normal pregnancy range, the mean values lie within the range yet at a lower level than the mean values for normal pregnancy. Four patients had subnormal values, Case 4 excreted 18.5 mg. at the 36th and 22 mg. at the 37th week when surgical induction resulted in the delivery of a normal baby weighing 5lb. 8oz. Case 10 has also a low pregnanediol excretion and Caesarean section at the 38th week resulted in the delivery of a live infant weighing only 4lb. 1oz.

TABLE 7. THE CLINICAL DETAILS OF 10 PATIENTS WHO HAD HYPERTENSION COMPLICATED BY TOXAEMIA

Hospital Number	Case Number	Baby's Weight	Placental Weight	Highest Blood Pressure mm/Hg.	Albumen g/1000 ml.	Oedema	Duration of Pregnancy Tox-aemia weeks	Labour Hours	Del-ivery weeks	Type of Delivery
8004	2	5 lb. 7oz.	1 lb. 2oz.	150/94	1.0	++	4	8	39	S.I. Spontaneous
5982	3	5 lb. 2oz.	1 lb. 3oz.	150/100	1.0	++	6	8	40	" "
8502	4	8 lb. 2oz.	1 lb. 7oz.	150/100	+	++	7	7	40	Spontaneous
11072	7	6 lb. 3oz.	1 lb. 2oz.	180/120	0.5	++	3	6½	37	S.I. Spontaneous
44845	9	5 lb.13oz.	14 oz.	150/90	+	+	4	4	40	" "
51735	10	6 lb. 1oz.	1 lb.4oz.	180/110	2.5	+	2	8	38	Spontaneous
Average		6 lb 2 oz.	1 lb.3oz.							
4363	1	4 lb.15oz.	1 lb. 5oz.	180/100	0.75g.	-	2	8	36	S.I. Spontaneous
12192	5	5 lb. 2oz.	1 lb. 2oz.	170/120	0.5 g.	+++	6	-	36	Caesarean Section.
5576	6	4 lb. 9oz.	15oz.	180/100	1.0 g.	+	6	-	35	" "
12425	8	3 lb.6oz.	13oz.	140/110	0.5 g.	++	4	-	36	Accidental haemorrhage 36 weeks Caesarean Section.
Average		4 lb.8oz.	1 lb. 1oz.						36	

S.I. = Surgical Induction

with a placenta of 14oz., these weights are low for this period of pregnancy. A low level of excretion was present in Case 11 but in spite of this a normal infant weighing 6lb. 4oz. was delivered. Foetal distress after a 13 hour labour developed in the fourth case (Case 12) where low pregnanediol excretion was detected. Thus of four patients with low pregnanediol excretion, two had an uneventful delivery, in one there was poor foetel growth and one had foetal distress in labour. In the nine patients who had normal levels no signs of placental insufficiency in the form of foetal distress or poor growth of the baby was detected.

It may be concluded that in this type of case when the pregnanediol excretion is low, placental insufficiency is more likely to be present than when the excretion level is normal.

2). Essential hypertension complicated by toxaemia.

Table 7 shows the clinical details of the 10 patients in this group. Six cases had toxaemia which responded to treatment and four had severe and fulminating toxaemia resistant to treatment. The

former cases were delivered at the 37th to 40th week of pregnancy, either after artificial rupture of the membranes or spontaneous labour, the remainder had their pregnancies terminated at the 35th or 36th week by Caesarean section (3 cases) or artificial rupture of the membranes (1 case).

Foetal distress occurred in Case 8 where an accidental haemorrhage at the 36th week precipitated a decision for immediate delivery by Caesarean section, the infant weighed 3lb. 6oz. at birth but cyanotic attacks occurred during the first four days of extra uterine death. Case 2 was delivered of a healthy infant weighing 5lb. 7oz. although infarcts were present over 50% of the placental surface. Macroscopic and microscopic examination showed the remainder of the placenta to be healthy.

The birth weights of the less severely toxaemic group ranged from 5lb. 2oz. to 8lb. 2oz. with an average of 6lb. 2oz. The placental weights were also within the normal range. In the severely toxaemic group, the birth weights varied from 3lb. 6oz. to 5lb. 2oz. (average 4lb. 8oz.) yet the placental weights

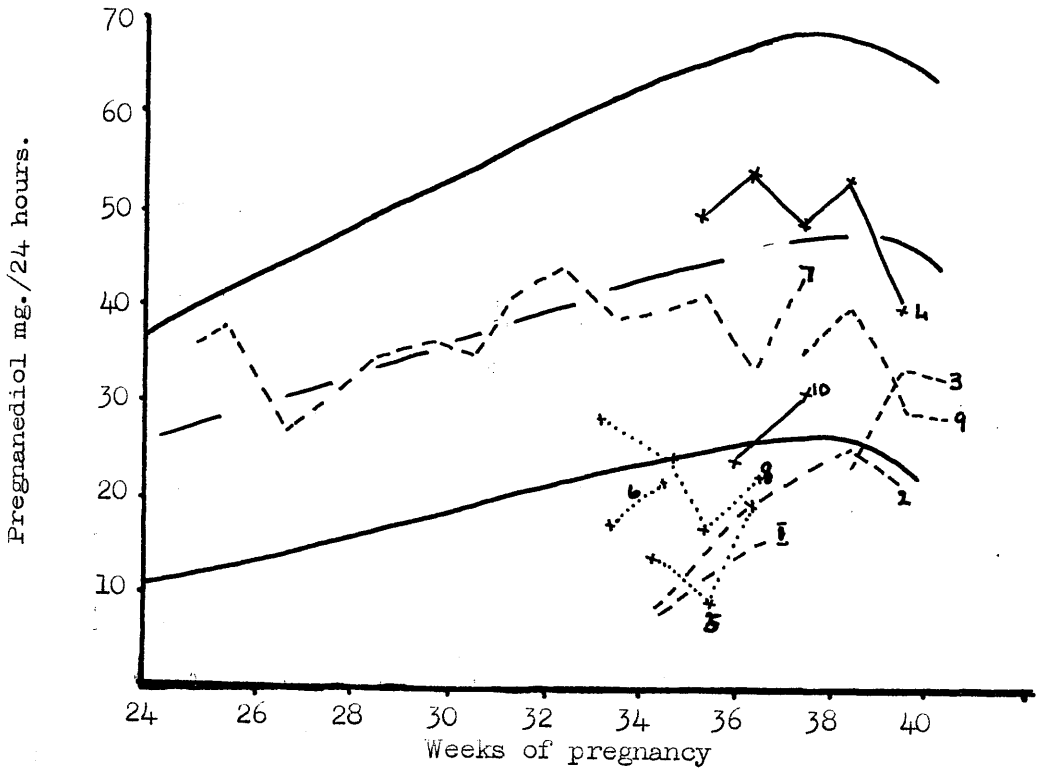


Fig. 6. THE PREGNANEDIOL EXCRETION, IN 10 CASES OF SEVERE PRE-ECLAMPTIC TOXAEMIA, WHO DELIVERED LIVE BABIES.

×——× Spontaenous delivery.

------ Surgical induction.

×·····× Caesarean section.

——— Normal pregnancy range.

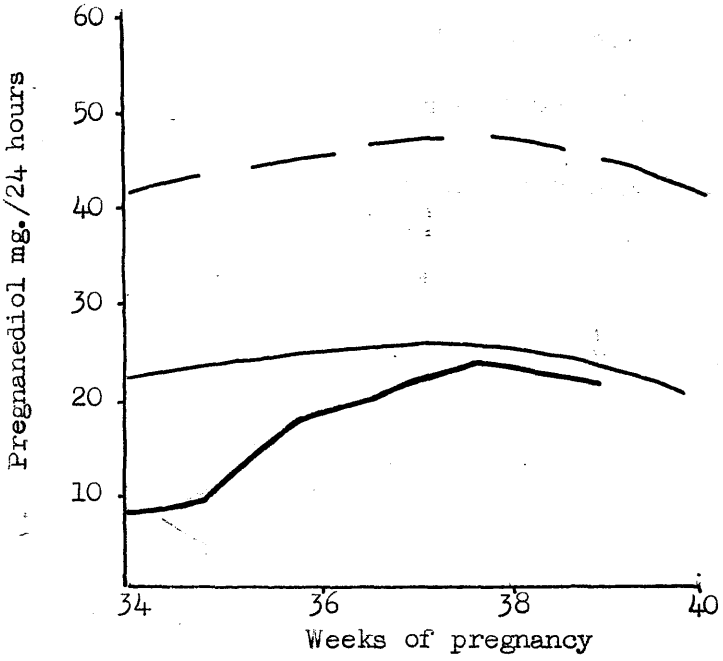


Fig. 7. Case 2. Table 7.

Baby 5lb. 7oz.

Surgical Induction (39 weeks).

Pregnanediol excretion.

——— Lower limit of the normal pregnancy range.

were still within the normal range. The average duration of pregnancy in this group was 36 weeks so it is evident that the babies' weights were subnormal for the gestation period.

The levels of pregnanediol excretions are shown in Fig. 6, individual graphs have been made for each patient. In three patients excretion was within the normal range and all three belonged to the less severe group of toxaemia. Two patients initially excreted subnormal amounts, but after the institution of treatment the excretion increased until normality was reached. These two patients belonged to the less severe group.

Four patients had a consistently low excretion, and of these, 3 belonged to the severe group whilst the fourth patient (Case 2) had 50% of the placental surface replaced by infarcts (Fig. 7b). Case 8 had a normal excretion but a gradual fall occurred which preceded and accompanied an accidental haemorrhage. The subnormal birth weights in this group correspond with subnormal levels of pregnanediol excretion.

In this group of patients it has been shown that the pregnanediol excretion varied with the severity of the toxaemia. In those cases where the toxaemia improved but did not disappear altogether, the pregnanediol excretion rose into the normal pregnancy range. In one of these cases the placenta was grossly infarcted but the functioning part was normal on histological examination and the pregnanediol excretion improved with rest and sedation; the baby was of normal weight when delivered.

In cases where the toxaemic symptons were severe and did not respond to treatment, the pregnanediol excretion was lower or a fall in excretion was noted. The babies survived although they were small for the period of gestation.

Discussion.

Th pregnancies associated with essential hypertension the pregnanediol excretion may give some indication of the rate of growth of the foetus and the functioning capacity of the placenta. In the majority of cases the excretion is within the normal pregnancy range but below the normal average, and the

foetal development and prognosis is good. In some cases the excretion is subnormal and in these foetal development may be slow.

When pre-eclampsia is superimposed on the hypertension, the picture of pregnanediol excretion is slightly changed. If the toxaemia is responsive to treatment, the foetal growth and the pregnanediol excretion levels remain or become normal. If however the toxaemia does not improve, the foetal growth is retarded and the pregnanediol excretion is subnormal or becomes so.

It seems that the pregnanediol assay may be of use to the clinician in the management of this type of case. The general policy in the treatment of the hypertensive pregnant woman is rest, sedation of the patient and possibly the administration of hypotensive drugs. These measures are specially enforced when toxaemia becomes superimposed on the hypertension. Normal pregnanediol excretion may serve to reassure the obstetrician, whereas subnormal or falling levels may serve as a warning that either foetal growth is slow or the placental reserve is poor, particularly in

Table 8. CLINICAL DETAILS OF 20 CASES OF FOETAL DEATH FROM TOXAEMIA OR ESSENTIAL HYPERTENSION.

Hospital Number	Case No.	Birth Weight	Placental weight	Blood Pressure mm/Hg.	Albumen g/1000 ml.	Oedema	Duration of Pregnancy when: Foetal Heart not heard	at Delivery (weeks)	Duration of labour (hours)	Type of delivery
8250	1	4lb.11oz.	1 lb. 2oz.	130/80	1.7	++	Neonatal death	36	14	S.I.Spontaneous
10427	2	3lb.	12oz.	180/95	2.0	++	" "	34		Caesarean Section
11069	3	1 lb.1oz.	8.5oz.	160/100	3.0	++	" "	34	24	S.I.Spontaneous
7934	4	1 lb.10oz.	7oz.	180/100	4.0	++	" "	29		Caesarean section
9267	5	3lb. 5oz.	9oz.	170/90	-	-	" "	35		" "
19062	6	1 lb.11oz.	5oz.	140/90	0.25	++	28	30		Spontaneous
9663	7	4lb. 4oz.	1 lb.5oz.	140/90	-	-	33	34		"
12084	8	5lb. 8oz.	14oz.	140/110	Trace	++	38	38		"
9147	9	5lb. 8oz.	1 lb.3oz.	180/110	++	+	38	38		"
9592	10	5lb. 6oz.	1 lb. 1oz.	150/90	-	-	40+	40+		"
44310	11	5lb. 2oz.	1 lb. 1oz.	140/90	0.5	+	36	36		"
1143	12	2lb.14oz.	8oz.	180/120	5.0	++	32	32		"
59769	13	1 lb.1oz.	8oz.	150/190	4.0	+++	26	26		"
10099	14	2lb.11oz.	14oz.	150/110	2.0	+	33	34		"
7055	15	2lb. 8oz.	9oz.	160/110	-	-	32	34		
6689	16	3lb. 6oz.	10oz.	170/100	-	-	33	34		"
7125	17	3lb. 2oz.	13oz.	170/100	2.0	-	34	35		"
11151	18	1 lb.4oz.	6oz.	190/140	8.0	++	24	28		"
8609	19	2lb. 7oz.	9oz.	180/110	2.5	+	34	34	9	Foetal heart stopped during labour
11714	20	2lb.14oz.	8oz.	180/120	5.0	++	32	32		Spontaneous
Average		3lb. 1oz.								

S.I. = Surgical Induction.

those cases where the toxaemia is resistant to treatment. The pregnanediol level may act as a guide for premature delivery in the difficult borderline cases.

C. THE PREGNANEDIOL EXCRETION IN 25 CASES OF PRE-ECLAMPTIC TOXAEMIA OR ESSENTIAL HYPERTENSION IN WHICH INTRA-UTERINE OR NEONATAL DEATH OCCURRED.

Pregnanediol estimations were made on 25 patients who had an intra-uterine or neonatal foetal death associated with pre-eclamptic toxaemia or essential hypertension. In 5 of these cases the additional complication of accidental haemorrhage occurred, the two groups have been studied under a separate heading.

Cases not complicated by accidental haemorrhage.

Table 8 shows the clinical details of the 20 patients in this group; all had hypertension, the blood pressure ranging from 190/140 mm.Hg. to 134/90 mm.Hg.: 14 had albuminuria with from 0.25 to 8.0 of albumen per 1000 ml. of urine and 14 had oedema.

Neonatal death occurred in 5 cases (1 to 5), the babies being delivered by Caesarean section in 3 cases and after a 14 and 24 hour surgically induced labour in 2. The birth weights at delivery were

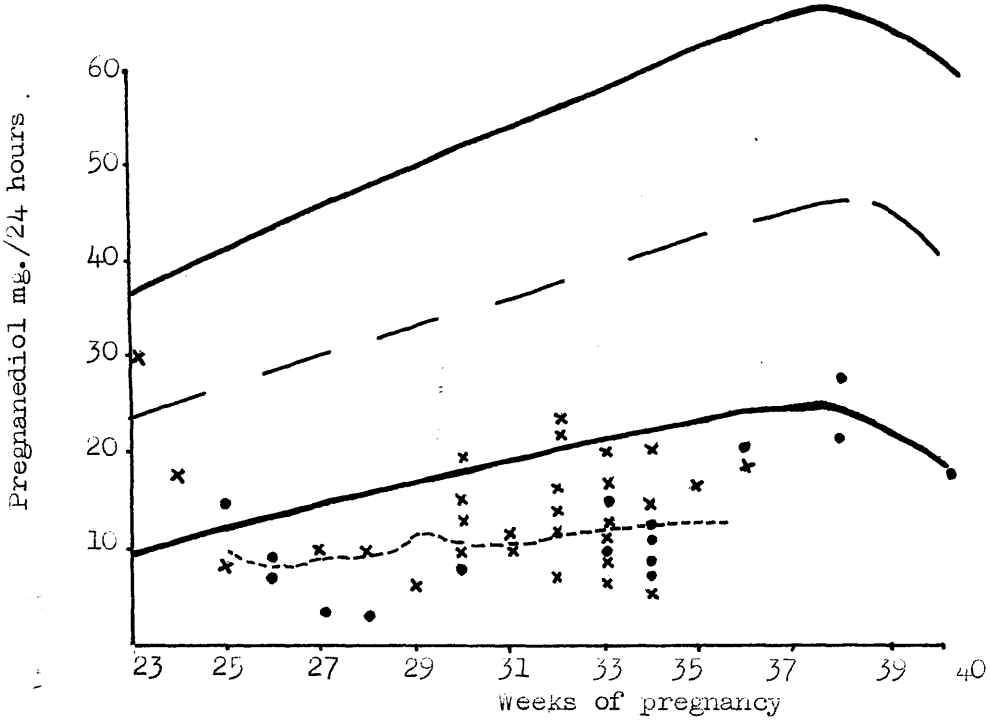


Fig. 8. A SCATTER DIAGRAM OF THE PREGNANEDIOL EXCRETION IN 20 CASES OF TOXAEMIA, WHERE THE FOETUS WAS EITHER STILLBORN OR DIED IN THE NEONATAL PERIOD.

××× Foetus still alive when estimation made

••• Foetal death occurred before estimation made.

——— Normal pregnancy range.

- - - - - - - - Average readings for toxaemia group.

TABLE 9. THE PREGNANEDIOL EXCRETION IN 5 CASES OF NEONATAL DEATH AND 15 CASES OF INTRA-UTERINE DEATH FROM TOXAEMIA

		Weeks of Pregnancy																
		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1.	Neonatal deaths.							15.4		17.0	17.4		18.2	20.0				
2.											8.0							
3.											8.1	7.6						
4.					10.0	10.0	6.5											
5.								20.4		22.1		21.4						
6.								8.5										
7.	The excretion after foetal death.										15.0							
8.																22.2		
9.																29.0		
10.																	21.0	
11.														21.0				
12.											11.2							
13.		8.4	9.8															
14.	The excretion before and after foetal death.										10.5	12.5						
15.										14.0	12.0	8.0						
16.										12.0	12.0	7.2						
17.									11.6	12.0	12.0	11.4						
18.		30.4	17.6	15.5	8.6	3.4	3.4											
19.	The arrow shows where death occurred.									24.0	20.0	17.4						
20.								9.0	11.2	6.8								

subnormal for a gestation period of 29 to 36 weeks. In the remaining 15 cases the foetus died within 1 and 4 weeks before delivery, the birth weights varied from 1 lb. 1oz. to 5lb. 8oz. with an average of 3lb. 1oz. This is a low for the average duration of pregnancy which was 34 weeks.

The pregnanediol excretion in this group of patients is shown on Table 9 and Fig. 8. In 7 of the cases studied (cases 6 to 12) it was not possible to obtain specimens before the foetal death but specimens were collected 1 to 14 days after the foetal death. It is assumed that these readings reflect the pre-death levels because in 5 cases (cases 13 to 17) where the pregnanediol excretion was determined before and 7 days after the foetal death, no appreciable fall was noted, and in the other 3 cases (18, 19 and 20) only a slight fall ranging from 2.0 to 4.4 mg./24 hours was noted.

The mean readings for the 20 patients were well below the lower limit of the normal pregnancy range. In 2 cases (18 and 19) the levels were initially normal but became subnormal before intra-uterine death

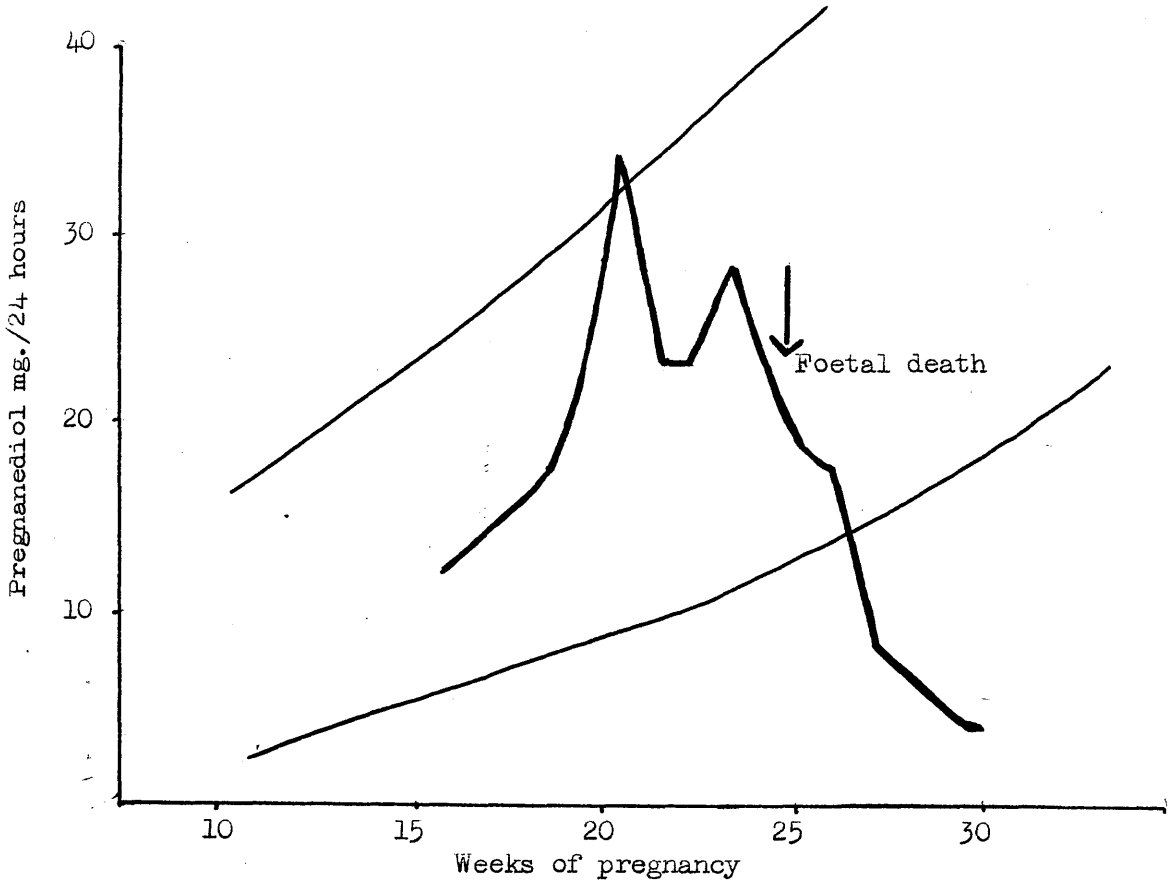


Fig. 9. Case 18. Table 9.

Baby 1 lb. 4oz. (Foetal Death at 26 weeks)

——— Normal pregnancy range.

occurred. Case 18 developed severe toxaemia at the 26th week of pregnancy and the baby died a few days later. The pregnanediol excretion had fallen in the 2 weeks preceding the foetal death (Fig. 9) and there was a slow steady fall to 3.0 mg./24 hours during the remaining 4 weeks before delivery. The placenta was fibrotic and histologically no evidence of functioning placental tissue could be found. The foetal death was associated with an improvement in the patient's general condition, the albuminuria improved and the blood pressure fell from 190/140 to 140/90 mm.Hg. In the 7 cases where pregnanediol determinations were made after intra-uterine death a subnormal level was found in 5, and a normal level in 2 cases. It is unfortunate that specimens were not obtained in these cases before the death because there may well have been a downward trend in the excretion.

Thus in these cases where the pregnancy was complicated by hypertension or toxaemia, and intra-uterine or neonatal death occurred, a significantly low level of pregnanediol excretion was found. This was associated with subnormal foetal development and again demonstrates

TABLE 10. THE PREGNANEDIOL EXCRETION IN 5 PATIENTS WHO HAD TOXAEMIA OF PREGNANCY COMPLICATED BY CONCEALED ACCIDENTAL ANTEPARTUM HAEMORRHAGE

Hospital Number	Case Number	Birth Weight	Placental Weight	Highest Blood Pressure mm/Hg.	Albumen gm/1000 ml.	Oedema	Time of Foetal Death	Duration of Pregnancy at Delivery.	Type of Delivery	Pregnanediol Excretion Before Accidental Haemorrhage	Pregnanediol Excretion After Accidental Haemorrhage
13860	1	1 lb.13oz.	8½ oz.	180/100	6.0	++	Neo-natal death 3 days	34	Spon-taneous	27.0	7.6
12999	2	3 lb. 4oz.	10 oz.	160/100	3.0	++	32	32	"	28.9	16.0
6679	3	3 lb. 4oz.	13 oz.	190/100	4.0	++	29	29	Surgical Induction.	23.8	7.4
9269	4	3 lb.	1 lb. 2 oz.	200/120	12.0	++	Neo-natal death 12 hrs.	30	Caesarean Section	38.5	29.2
4341	5	3 lb. 6 oz.	10oz.	155/100	2.0	++	33	34	Spon-taneous	30.0	9.1

Patients 1, 2 and 3 had an eclamptic fit preceding the accidental haemorrhage.

that low levels of pregnanediol excretion may indicate that the foetus is developing slowly and that the placental reserve is already reduced. A further reduction may occur if the maternal toxaemia does not respond to treatment or if a further deterioration in the maternal condition occurs.

Cases not complicated by accidental ante-partum haemorrhage.

Of the 5 patients in this group the clinical details are shown in Table 10; all patients were severely toxaemic and three had eclamptic fits preceding the haemorrhage. One was delivered by Caesarean section, 2 had labour induced and 2 delivered spontaneously. Three of the infants were stillborn and 2 died in the neonatal period. The average birth weight was 2lb. 15oz. This is low for an average duration of pregnancy of 32 weeks. The placentae were examined and the diagnosis of Accidental Ante-partum Haemorrhage was confirmed.

The pregnanediol excretion before and after the haemorrhage is shown in Table 10. In all cases the level was normal before the haemorrhage and in 4 cases

a subnormal level was found on the following day. In the 5th case the level fell significantly but not to a subnormal level. These results accord confirmation of the fact that the pregnanediol levels are related to the amount of functioning placental tissue remaining after the haemorrhage and that a fall in pregnanediol excretion indicates a decrease in placental function and consequently reflects incipient danger to the foetus.

DISCUSSION.

It is a curious fact that there is no change of pregnanediol excretion preceding eclampsia or accidental haemorrhage in this small series. This seems to be at variance with a statement made in an earlier section, namely that the more severe type of toxaemia which is resistant to treatment is associated with low pregnanediol levels. However in these cases the onset of the toxaemia was sudden and the duration of the symptons before the accidental haemorrhage ranged from 1 - 3 weeks and of the factors which contribute most to the reduction in placental function the duration of the toxaemia is one of the more important.

D. THE PREGNANEDIOL EXCRETION IN PREGNANCY ASSOCIATED WITH CHRONIC NEPHRITIS.

INTRODUCTION.

In pregnancy associated with chronic nephritis the prognosis for the foetus is poor. The babies are small for the duration of pregnancy and the placentae small and insufficient. Pigeaud and Bureiul (1947) showed that in these cases there was a relationship between the placental weight and the pregnanediol excretion in that when the placenta weighed under 1 lb., low levels of excretion were found and when it was between 1.5 and 1.9 lb. a higher excretion was present. They suggested that the foetal prognosis was worse when the impaired renal function was associated with hypertension. Trolle (1955) maintained that the presence of albumen in the urine in these cases, could give rise to fallacious results the albumen inducing a solid emulsion and making recovery of the pregnanediol difficult.

Pregnancy complicated by chronic renal disease is a rare condition but 5 patients were admitted to

TABLE 11. THE CLINICAL DETAILS AND PREGNANEDIOL EXCRETION IN 5 PATIENTS WITH CHRONIC RENAL DISEASE

Hospital Number	Case Number	Birth Weight	Placental Weight	Highest Blood Pressure mm/Hg.	Albumen g/1000 ml.	Blood Urea mg/100ml	Casts	Outcome of Pregnancy	Duration of Pregnancy at Delivery	Type of Delivery.
58066	1	4 lb. 4oz.	13oz.	200/100	1.4	60	Granular ++	Died 6 hr.	34	Caesarean Section
13874	2	3 lb.1oz.	2 lb. 9oz.	160/100	8.0	29	Hyaline + Granular +	Died 2 hr.	30	Spontaneous
14513	3	1 lb.2oz.	6oz.	170/130	4.5	52	Granular ++	Died 5 hr.	24	Hysterotomy
7712	4	5 lb.12oz.	1 lb.	160/100	3.0	39	Granular +	Survived	39	Caesarean Section
49693	5	2 lb. 5oz.	8oz.	190/110	4.0	34	Hyaline + Granular ++	Died 24 hrs.	34	Caesarean Section

PREGNANEDIOL EXCRETION

Case Number	Weeks of Pregnancy																	
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1	2.0	7.0	5.8	6.0	-	5.3	4.5	8.9	5.3	2.3	6.4	2.3	3.5					
2								23.0	17.0									
3	1.0	0.8	1.2															
4						12.0	16.0						18.0	15.7	18.2	19.0	21.0	18.0
5										6.3	6.7							

hospital during the time when this work was in progress, 4 had chronic nephritis and one chronic pyelonephritis with marked impairment of renal function.

RESULTS.

The clinical findings are shown in Table II. The blood urea varied from 29 to 60 mg. per 100 ml, albumen from 1.4 to 8 gm. per 1000 ml. and granular casts were found in all cases. The pregnancies were complicated by hypertension in all cases, the blood pressures varying from 160/100 to 200/100 mm.Hg.

The pregnancy in case 3 was terminated by hysterectomy at the 24th week because the patient's general condition was deteriorating: the blood pressure was rising and the albuminuria increasing. The baby was 1 lb. 2oz. in weight and lived for 5 hours. The placenta weighed 6oz., it was unhealthy; there was evidence of an accidental haemorrhage and histological evidence of insufficiency. The pregnanediol excretion was abnormally low (1.0 mg./ 24 hours at the 21st week of pregnancy) and it remained low during the 3 weeks before delivery. In the first

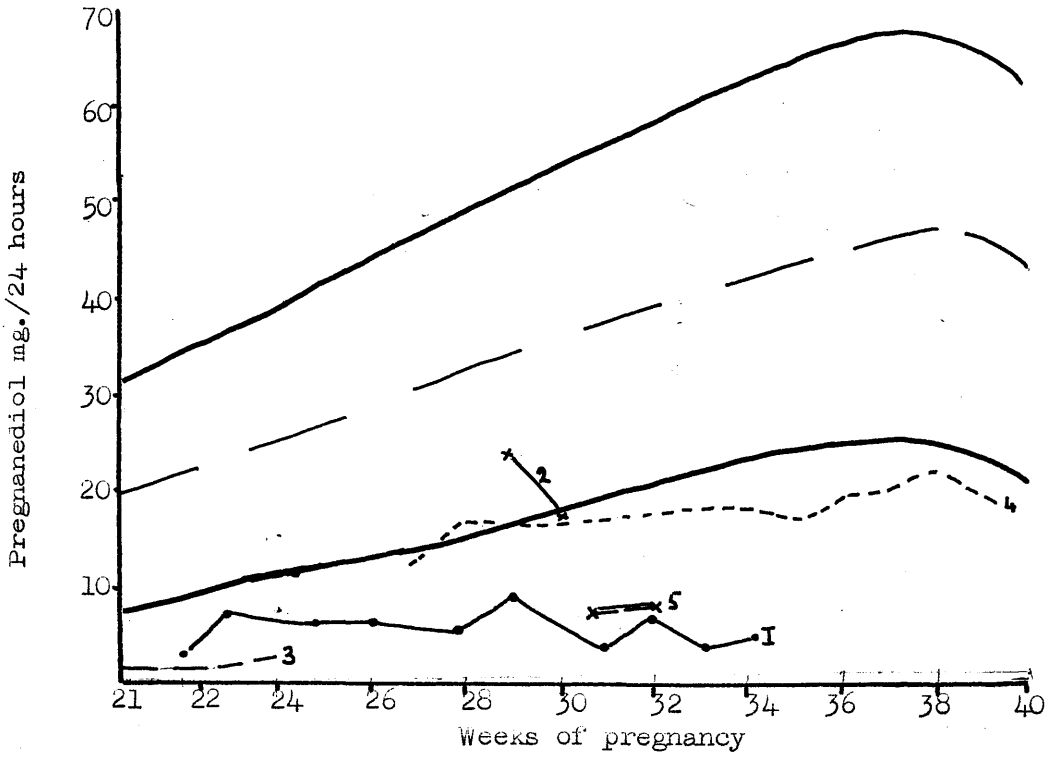


Fig. 10. THE PREGNANEDIOL EXCRETION IN 5 CASES OF PREGNANCY ASSOCIATED WITH CHRONIC RENAL DISEASE. (Table 11).

Pregnanediol excretion.

Normal pregnancy range.

24 hours following delivery, the patient excreted 1.5 mg./24 hours (Fig. 10) and the excretion was only 0.5 mg. on the 4th day of the puerperium. It is difficult to believe that a baby could grow to 1 lb. 2oz. with a blood progesterone level indicated by a pregnanediol excretion which was at a similar level to that found in the proliferative phase of the menstrual cycle, yet a functioning placenta must have been present.

Case I was somewhat similar to the above. The patient was delivered by Caesarean section at the 34th week of pregnancy, of a baby weighing 4lb. 4oz., which lived for 6 hours. The placenta was 13oz. in weight, and there was an area of accidental haemorrhage and histological evidence of insufficiency. The pregnanediol excretion (Fig.10) was again abnormally low (3 to 8 mg.) throughout the pregnancy, the level being 3.5 mg. before delivery. In the 14 days after delivery the level varied from 1.2 to 0.4 mg./24 hours.

In case 5, Caesarean section was performed at the 33rd week of pregnancy and the baby which was only 2lb. 5oz. in weight lived for 24 hours. The

placenta weighed only 8oz. and about half of its functioning area was replaced by an infarct. The pregnanediol excretion in this case was low (6 - 7 mg.) which was in agreement with the low birth weight of the baby and the poor functioning capacity of the placenta.

The duration of pregnancy at delivery in case 4 was 39 weeks. The baby which was 5lb. 12oz. in weight survived, after delivery by Caesarean section. The placenta weighed 16oz. and there was histological evidence of insufficiency. The pregnanediol excretion was maintained at a level of from 16 to 21 mg./24 hours from the 27th to the 39th week of pregnancy. This is below the normal pregnancy range. In this case the low birth weight, the placental histology and the pregnanediol excretion were in agreement.

Rhesus immunisation as well as chronic nephritis was present in case 4. The baby, a hydrops, died within 2 hours of birth, and the placenta, which was erythroblastatic, weighed 2lb. 9oz. The pregnanediol excretion was within the

normal pregnancy range at the 29th week of pregnancy but it fell to 17.0 mg./24 hours before delivery at the 30th week. It was the highest pregnanediol excretion noted in this series and corresponded with an enlarged and erythroblastatic placenta.

DISCUSSION.

Low levels of pregnanediol excretion were found in 4 patients whose pregnancy was complicated by impaired renal function.

In one of these cases the excretion was maintained at a level just below the lower limit of the normal pregnancy range and the baby was small for a pregnancy of 39 weeks gestation (case 4). Histological evidence of placental insufficiency was found. This is in agreement with the results already reported in the section on toxaemia of pregnancy. The findings in case 5 were similar; the pregnanediol excretion was 6.3 mg./24 hours and the baby weighed only 2lb. 5oz. at the 33rd week of pregnancy. The placenta was grossly infarcted.

In the other 2 cases abnormally low levels of

excretion were detected, and although placental insufficiency was probably present, it is difficult to believe that live babies can be produced with a functioning placental capacity inferred by a pregnanediol excretion of only 1.0 or 3.5 mg./24 hours. It is possible that in these cases the pregnanediol excretion was not a true index of placental function and that the abnormally low levels were caused by failure of the kidneys to excrete the total amount of pregnancy formed by the placenta. If however the pregnanediol had been accumulating in the blood, then it should have been excreted in the puerperium when the renal function improved. This did not occur in the 2 cases studied. These cases were however only studied for periods of 4 and 14 days and the kidney function may not have recovered sufficiently during that time for the accumulated pregnanediol to be excreted. Pregnanediol before it is excreted by the kidneys is conjugated either in the liver or the kidney to pregnanediol glycuronide, which is water soluble. It is possible that if the kidney is the site of conjugation then in cases where the kidney function is impaired the conjugation is

incomplete, and the kidneys may be unable to excrete pregnanediol which is insoluble in water. It may then be excreted by the alimentary tract or further metabolised.

In the last case the pregnanediol excretion was normal initially and then a slight fall occurred. The placenta was erythroblastotic and the cause of foetal death was Rhesus immunisation and not placental insufficiency.

These patients all had albuminuria and it was suggested by Trolle (1955) that this could cause an incomplete recovery of pregnanediol because solid emulsions, which are difficult to disperse, form in the toluene extracts of urines containing albumen. In the work reported here, the emulsions were broken down by adding 2 drops of "Teepol" (Shell Chemicals, London) to the extracts followed by washing with 25 ml.N/NaOH containing 25% NaCl.

This technique was used on 12 urines containing from 0.05 to 3 g. per 1000 ml. of albumen, to which 0.4 mg. of pure pregnanediol had been added to 150 ml. of urine. The recovery rates varied from

Table 12. THE RECOVERY OF PURE PREGNANEDIOL FROM URINES CONTAINING ALBUMEN.

Clinical Conditions	Albumen	Pregnanediol excretion	Duration of Pregnancy	Recovery
Chronic pyelonephritis	0.3gms.	7 mgs.	29 weeks	98%
Chronic nephritis	1.5 gms	18.9 mgs.	25 weeks	93%
Toxaemia	0.5 gms.	17 mgs.	29 weeks	100%
Chronic Nephritis	0.22gms.	2.0 mgs.	23 weeks	87%
Hypertension	0.05gms.	24.4 mgs.	30 weeks	100%
Toxaemia	0.5 gms.	10.2 mgs.	34 weeks	100%
Toxaemia	0.5 gms.	28.4 mgs.	29 weeks	88%
Toxaemia	0.5 gms.	10.4 mgs.	30 weeks	91½%
Toxaemia	0.55gms.	7.0 mgs.	30 weeks	100%
Toxaemia	0.2 gms.	9.6 mgs.	28 weeks	80%
Chronic Nephritis	3.0 gms.	4.16 mgs.	20 weeks	91%
Chronic Nephritis	0.75gms.	8.9 mgs.	24 weeks	90%

80% to 100% (Table 12), these are satisfactory recoveries for this method. This experiment only shows that free pregnanediol can be recovered from urines containing albumen and if the pregnanediol glycuronide is bound to the protein molecule some may be lost during extraction.

DISCUSSION AND PROPOSALS FOR FUTURE WORK.

This investigation was undertaken to establish the value of the urinary pregnanediol assay in the management of cases of toxaemia of pregnancy. The results obtained in the 75 cases have been grouped according to the patients' symptons, their response to treatment and the outcome of the pregnancy.

In the cases where the toxaemia was of the less severe type of where there was a good response to treatment (rest and sedatives) the pregnanediol excretion was either within the normal pregnancy range or a subnormal level was found which became normal when the patient's toxaemia or hypertension improved. These patients were delivered between the

37th and 40th week of pregnancy and the babies which were of normal weight survived.

Subnormal or falling levels of pregnanediol excretion were found in the patients who had a severe type of toxaemia, but who were delivered of live babies. The excretion levels in these cases appeared to give some indication of the foetal prognosis, because although duration of pregnancy at delivery was earlier than in the less severe group, the babies were of subnormal weight for the gestation period. In the group who had stillbirths or neonatal deaths, in some cases very low levels of pregnanediol excretion were found (6 to 10 mg./24 hours) and the average excretion of the whole group was below the lower limit of the normal pregnancy range.

The results in these cases showed that, in cases of hypertension or toxaemia, the pregnanediol excretion gave some indication of the response to treatment, and of the final outcome of the pregnancy. In the treatment of severe toxaemia the pregnancies are often not terminated before the 36th week because of the high foetal mortality from prematurity. In

some of the cases presented here, intra-uterine foetal death occurred before the 36th week of pregnancy and subnormal levels of pregnanediol excretion were found before the foetus died. The cause of the foetal death was probably anoxia caused by a gradual reduction in the functioning capacity of the placenta. Potter & Adair (1939) performed post mortem examinations on a large number of stillbirths and neonatal deaths and found that anoxia was one of the factors in which contributed to the high foetal mortality from toxaemia of pregnancy. In cases, where a subnormal level of pregnanediol excretion or a significant fall in excretion is found, and clinically the baby does not appear to be growing, earlier delivery may prevent the foetus from being subjected to a prolonged period of anoxia and the foetal prognosis may be improved.

In this type of case it would be useful to correlate the cord blood oxygen saturation and the level of pregnanediol excretion. Clemetson & Churchman (1953), Walker & Turnbell (1953) and Walker (1954) showed that in toxaemia of pregnancy

the cord blood oxygen saturation is reduced. Thus, if a relationship between the level of oxygen saturation, the pregnanediol excretion, and the fate of the foetus was found in cases of severe toxaemia it might be possible to establish the true pathological levels of pregnanediol excretion which indicate that the placental function is insufficient for the foetal requirements.

The factors which appear to have most influence on the pregnanediol excretion are the severity and the duration of the toxaemia and the maternal response to treatment. In 5 cases of fulminating toxaemia of short duration (1 to 2 weeks) where the babies died from the effects of accidental haemorrhage the pregnanediol excretion was normal before, but subnormal after, the haemorrhage.

It has been assumed that the low pregnanediol levels found in some cases of toxaemia may reflect the functioning capacity of the placenta and it is possible to correlate the pregnanediol excretion levels with low birth weights and with the foetal mortality. There are however 2 other possible explanations for

the low levels found, namely, that the kidney function was disturbed; or that when albumen is present in the urine, there is an incomplete recovery of pregnanediol. In 4 patients who had chronic renal disease, 2 excreted pregnanediol at the rate of 6.3 and 21 mg./24 hours and 2 at 1.5 and 3.5 mg./24 hours, the babies' weights were subnormal in all 4 cases and there was histological evidence of placental insufficiency. In these cases both a diminution in the progesterone production and the disturbed kidney function may have been responsible for the subnormal pregnanediol excretion. This could be proved if blood and urinary pregnanediol levels could be compared but there is as yet no satisfactory method of estimating the blood pregnanediol (Klopper 1956, Personal communication). It was decided that if pregnanediol clearance tests were performed on patients with low levels of pregnanediol excretion, and the rate of clearance compared with the normal pregnancy rate, the results obtained might show whether the injected pregnanediol was being retained. It is difficult to obtain pure pregnanediol commercially but 2 gms. of pure pregnanediol was prepared from

200 litres of late pregnancy urine. A fine suspension of pregnanediol (50 mg.) in 2 ml. of ethyl oleate was injected into 3 members of the staff but no pregnanediol was recovered in the urine during the 7 days following the injection. Guinea pigs were then injected with a similar preparation of pregnanediol and 3 days later 75% of the pregnanediol was recovered from the site of the injection. The pregnanediol was probably not absorbed, because it is insoluble in water but if sufficient quantities of pure pregnanediol glycuronide were extracted and purified, this being water soluble would probably be more suitable for the clearance test. The results of this test might help to prove what factors are responsible for the low pregnanediol excretion levels found in the toxaemias of pregnancy.

When pregnanediol was added to 12 urines containing albumen, satisfactory recoveries of 80% to 100% were obtained. It was thus assumed that the albumen present in the urine was not responsible for the subnormal levels of pregnanediol excretion found in toxaemia of pregnancy. Moreover in some cases of

essential hypertension where albuminuria was not present low levels of pregnanediol excretion were found.

In some cases of toxaemia or hypertension where subnormal levels of pregnanediol excretion are initially found, a rise to normal levels may occur in response to treatment. In toxaemia and hypertension the blood supply to the uterus is reduced by spasm of the spiral arteries and this reduces the placental blood supply and may cause placental insufficiency. In those cases where a response to treatment is noted, the placental blood supply and function is probably improved, and this appears to be reflected by an increase in the pregnanediol excretion. This may be useful in determining the value of new forms of treatment in these conditions.

Summary and Conclusions.

The urinary pregnanediol assay appears to be of value in the management of cases of toxaemia of pregnancy. Normal levels have been found in cases where the foetal prognosis is good, and subnormal

levels in cases where the toxaemia is complicated by intra-uterine or neonatal foetal death. A falling level below the normal pregnancy range, appears to be significant if the fall is maintained over a period of 2 or more weeks or if the fall is associated with a deterioration in the maternal condition.

The low levels of pregnanediol excretion found in toxaemia of pregnancy may not be caused by a fall in progesterone production. They may be due to either failure of the kidneys to excrete the pregnanediol produced or the incomplete recovery of pregnanediol from urines containing albumen. In 2 cases of chronic renal disease abnormally low levels of pregnanediol excretion were found, but in the cases of toxaemia good correlation was found between the pregnanediol excretion level, the birth weight and the placental histology. Satisfactory recoveries were obtained when pregnanediol was added to urines containing albumen and in cases of hypertension where albuminuria was not present low levels of pregnanediol excretion were found.

It may thus be concluded that the pregnanediol excretion can reflect placental function in cases of toxaemia and that it may be used to assist in the management of cases where there is doubt about the foetal prognosis. This assay may also be of value in assessing the effects of treatment, i.e. by hypotensive drugs, in cases of severe toxaemia and hypertension in pregnancy.

PART B.

THE PREGNANEDIOL EXCRETION IN CONDITIONS ASSOCIATED WITH FOETAL DEATH IN LATE PREGNANCY.

It has been shown that in toxaemia of pregnancy subnormal levels of pregnanediol excretion may be found in those cases complicated by foetal death. It was decided to investigate patients who had foetal deaths due to other late pregnancy disorders. The investigation was extended to include patients with a history of an unexplained stillbirth or neonatal death in a previous pregnancy and who were again pregnant.

The investigation is divided into 4 parts according to the associated condition :

A) Antepartum haemorrhage.

B) Diabetes mellitus.

C) Rhesus immunisation, including cases of unexplained foetal death.

D) Bad obstetric history.

Table 1. THE CLINICAL DETAILS AND THE PREGNANEDIOL EXCRETION IN 10 CASES OF PLACENTA PREVIA.

Hospital Number	Case No.	Birth Weight	Duration of pregnancy at delivery (weeks)	Weeks of pregnancy 32	33	34	35	36	37	38	39	Type of delivery Remarks
5701	1	7lb. 5oz.	39							28.0	31.3	Caesarean section
8558	2	4lb. 8oz.	38				43.2	27.6	28.0			Caesarean section
8192	3	(5lb. 7oz. (6lb. 4oz.	39				47.0	52.0	47.0	41.6		Spontaneous (Twins)
11880	4	6lb. 2oz.	37		38.0		34.0	55.0	53.0			Caesarean section
4724	5	6lb. 6oz.	36		32.0		34.3	47.5				Spontaneous
27948	6	4lb.13oz.	38				34.8	44.8				Caesarean section
10514	7	5lb. 7oz.	35		34.4	38.2						Caesarean section
10235	8	8lb.15oz.	37			47.2	53.4	44.6	44.0			Surgical Induction
6126	9	6lb. 5oz.	35		65.0	28.5	45.6					Caesarean section
1629	10	6lb. 4oz.	36	31.8	50.4	38.0	42.0	40.0				
Average		6lb.12oz.			44.0	37.7	41.8	44.5	43.0	34.8	31.3	

A. THE PREGNANEDIOL EXCRETION IN ANTE-PARTUM HAEMORRHAGE.

INTRODUCTION.

Placenta previa and accidental haemorrhage not associated with toxaemia or hypertension are two of the principal causes of vaginal bleeding in late pregnancy. In both these conditions there is partial separation of the placenta from the uterine wall and this may reduce the area of functioning placental tissue.

Patients admitted with a history of vaginal bleeding in the later weeks of pregnancy had pregnanediol estimations made at regular intervals until delivery. There were 20 cases investigated, the final diagnosis was placenta previa in 10 and in the other 10 cases a diagnosis of accidental haemorrhage was made.

RESULTS.

Placenta Previa Group.

The clinical details are shown on Table I In one patient twin babies were born at the 39th

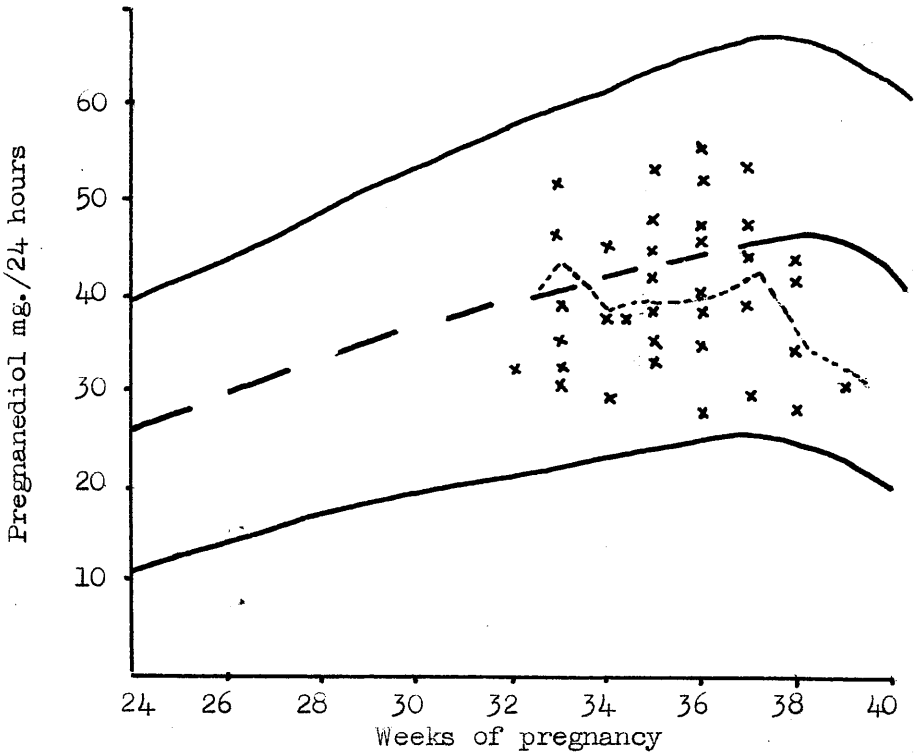


Fig. I. A SCATTER DIAGRAM OF THE PREGNANEDIOL EXCRETION LEVELS, IN 10 CASES OF PLACENTA PREVIA (Table I).

——— Normal pregnancy range.

- - - - - Average readings for the Placenta Previa group.

week of pregnancy, in the other cases living babies were delivered between the 35th and 39th week. Caesarean sections were performed in 7 cases and 3 cases had spontaneous vaginal deliveries. The birth weights varied from 4lb. 8oz. to 8lb. 15oz., the average being 6lb. 12oz. which is normal for an average duration of pregnancy of 37 weeks although 2 babies were small for the relevant gestation period (cases 2 and 6) and one of these babies died in the neonatal period (case 2).

The pregnanediol excretion Fig. 1., Table 1 was within the normal pregnancy range but the mean values were below the mean values for normal pregnancy. It is of interest to note that in case 2 a significant and sustained fall within the normal pregnancy range occurred at the 36th week of pregnancy. This has been observed in normal pregnancy but in this case the baby's weight was subnormal and neonatal death occurred. The excretion level in the case of twin pregnancy was not higher than in normal pregnancy in spite of the excessive weight of the placenta ($2\frac{1}{2}$lb.) and the combined weights of the babies (11 lb. 11oz.).

Table 2. THE CLINICAL DETAILS AND THE PREGNANEDIOL EXCRETION IN 10 CASES OF ACCIDENTAL ANTEPARTUM HAEMORRHAGE.

Hospital No.	Case No.	Birth Weight	Duration of pregnancy at delivery (weeks)	Weeks of pregnancy: 27	28	29	30	31	32	33	34	35	36	37	38	39	40	Type of delivery	Date of Baby
7417	1	7lb. 6oz.	40														40.6	Spontaneous	Alive
54229	2	2lb.12oz.	32	16.0	16.0	18.0	14.0	12.3	13.0									Caesarean section	Alive
5402	3	4lb.11oz.	37											11.5 ↓				S.I. Spontaneous	Stillborn
5124	4	8lb.	38									31.2	28.5	39.5	48.0			" "	Alive
6123	5	2lb.13oz.	38							31.9	29.0	20.8	24.0	14.9				" "	Alive
6131	6	5lb.	39									17.8	19.4	17.8	25.8	23.3		Spontaneous	Stillborn
6809	7	3lb.12oz.	33				12.5 ↓	10.5										S.I. Spontaneous	Stillborn
4560	8	4lb.12oz.	37	20.5	29.6	23.2	23.0	27.6	23.8	28.7	39.3	36.1	27.9	24.3				Caesarean section	Alive
10900	9	6lb.	36									29.1	30.0					Spontaneous	Alive
13172	10	4lb.11oz.	38												11.6 ↓			"	Stillborn
Average		4lb.15oz.	37															S.I. = Surgical Induction	

↓ Indicates when the foetus died.

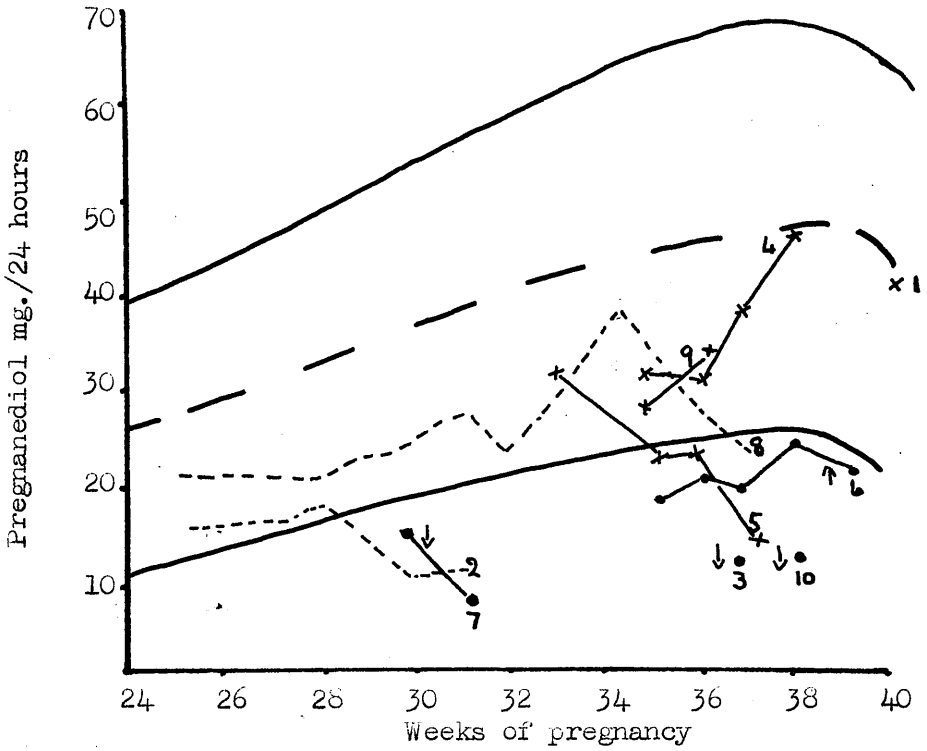


Fig.2. THE PREGNANEDIOL EXCRETION IN 10 CASES OF ACCIDENTAL ANTEPARTUM HAEMORRHAGE (Table 2).

------- Live birth Caesarean section.

x——x Live birth Spontaneous delivery.

•——• Foetal death. ↓ Time of foetal death.

——— Normal pregnancy range.

Accidental Haemorrhage Group.

The clinical details of the 10 patients are shown on Table 2. Living babies were delivered vaginally in 4 patients, by Caesarean section in 2 patients and 4 patients had stillbirths. The average birth weight for this group was 4lb. 15oz. which is low for an average gestation period of 37 weeks. The diagnosis of concealed and revealed accidental haemorrhage was confirmed in all cases by examination of the placenta.

The pregnanediol excretion levels are shown on Table 2 and Fig. 2. The levels were normal in 2 cases (4 and 9); both delivered live babies which were normally developed for the relevant gestation period. In 3 cases (2, 5 and 8) the pregnanediol excretion started to fall at the 27th (case 2) and the 33rd weeks (cases 5 and 8) and it had dropped below the normal pregnancy range before delivery. The normal pregnancy fall occurs between the 36th and 40th week. Live babies were delivered in these cases, 2 by Ceasarean section (cases 2 and 8) and one spontaneously after a short labour (case 5).

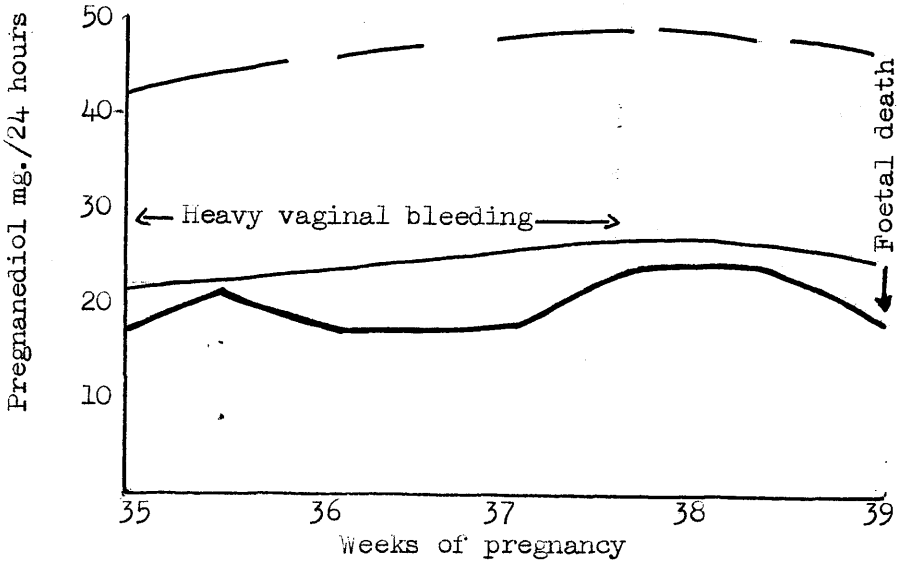


Fig. 3. Case 6. Table II (Accidental Haemorrhage)
Baby 5lb. (died 24 hours before delivery)

——— Pregnanediol excretion.

——— Lower limit of the normal pregnancy range

The babies survived although their birth weights were subnormal.

In the other 4 cases (3, 6, 7 and 10) stillborn babies of low weight for the gestation period were delivered and the pregnanediol excretion was again subnormal.

Thus, low pregnanediol levels can be correlated with slow foetal growth in the majority of these cases and persistantly subnormal levels with a poor foetal prognosis. In view of these findings it is suggested that in cases of accidental antepartum haemorrhage a falling pregnanediol excretion, before the 36th week of pregnancy, may be used as an indication for early delivery, either vaginally or by Caesarean section and in cases where subnormal levels persist labour should be induced at an early date to avoid the incidence of intra-uterine foetal death.

DISCUSSION.

Haemorrhage in the later weeks of pregnancy from a low implantation of the placenta does not affect the pregnanediol excretion. The excretion

in 10 cases of placenta previa was within the normal pregnancy range and the average birth weight of the babies, 6lb. 5oz., was normal for an average duration of pregnancy of 37 weeks.

In the accidental haemorrhage group the pregnanediol excretion was either inititally below or fell below the normal pregnancy range in 7 cases and in 4 of these cases the babies were stillborn. The average weight of the babies was low, 4lb. 13oz., for an average duration of pregnancy of 37 weeks. Thus in this group the pregnanediol excretion was lower than in the placenta previa group and the babies were smaller although the duration of pregnancy was the same in both groups. There were more stillbirths in the accidental haemorrhage group. Thus, in placenta previa, although the placenta becomes partly separated from the uterine wall the separation is not due to an inherent fault in the placental vascularity or function and the pregnanediol excretion is often maintained at a normal level after the separation has occurred. In accidental haemorrhage the placental function is reduced, because the

placenta is separated from the uterine wall by a retroplacental clot and the pregnanediol excretion may fall to subnormal levels after the haemorrhage occurs. In 3 of the cases presented here the fall in excretion occurred between 27th and 33rd weeks and placental insufficiency may have been present before the final haemorrhage at the 31st, 36th and 37th weeks of pregnancy.

CONCLUSIONS.

The pregnanediol excretion may give some guide to placental function in cases of antepartum haemorrhage. A fall or a sustained low level of excretion may indicate that the foetus is in danger and if the pregnancy is sufficiently advanced for the baby to lead an independent existance delivery should be precipitated.

In accidental haemorrhage there is a reduction in the area of functioning placental tissue, and the baby may die from malnutrition. In this type of case the pregnanediol excretion is subnormal and the birth weights are low and the incidence of intra-uterine foetal death is increased.

B. THE PREGNANEDIOL EXCRETION IN DIABETES AND PREGNANCY.

INTRODUCTION.

The management of the pregnant diabetic is a difficult problem because in about 10% to 30% of cases the baby is stillborn or dies in the neonatal period. It has been shown that there is a fall in the pregnanediol excretion preceding intrauterine death in a number of cases (White & Hunt, 1940, and Smith & Smith 1947). These workers presupposed that the low pregnanediol excretion was due to insufficient progesterone production. Working on these results White (1949) and Smith & Smith (1953) showed that the foetal survival was increased if replacement hormone therapy in the form of stilboestrol and progesterone was administered throughout pregnancy. Gray (1954) and Rolland (1954) however found that in diabetic pregnancy the pregnanediol excretion was within the normal range and that the assay could not be used as a guide to foetal prognosis.

PLAN OF INVESTIGATION.

Nineteen diabetic patients, all having insulin therapy, were kept under observation throughout pregnancy. The diabetes was diagnosed during pregnancy in 2 cases and between 1½ and 10 years before the pregnancy in the remaining seventeen. The duration of pregnancy at delivery varied from 34 to 40 weeks and the babies' weights from 5 to 7 pounds, there were 4 stillbirths. Three patients had hypertension in early pregnancy and intrauterine foetal death occurred in one of them. Two patients developed pre-eclamptic toxaemia during pregnancy but both delivered live babies. The other 3 stillbirths, all the result of antepartum foetal death, occurred in patients who had no complications other than diabetes. In 15 cases live babies were delivered either by Caesarean Section (9 cases) or spontaneously after surgical rupture of the membranes (6 cases). Pregnanediol estimations were made at regular intervals after the 28th week of pregnancy in all cases.

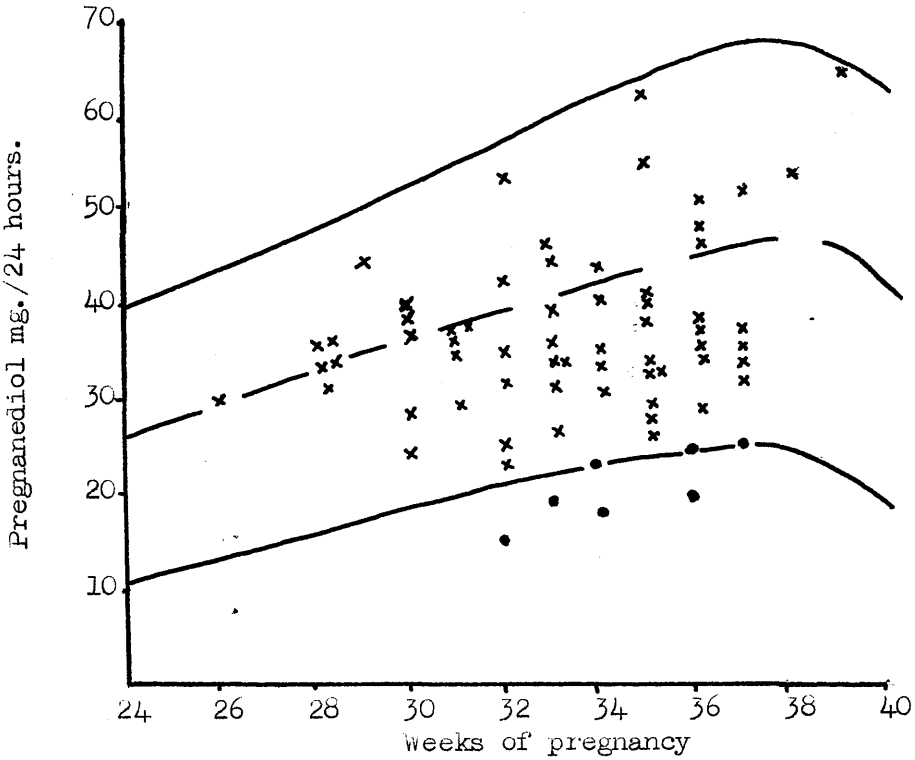


Fig. 4. A SCATTER DIAGRAM OF THE PREGNANEDIOL EXCRETION LEVELS, IN T5 PATIENTS WHO HAD DIABETES AND DELIVERED LIVE BABIES.

×× Uncomplicated diabetes.

•• Diabetes complicated by hypertension or toxaemia.

Table 3. THE CLINICAL DETAILS AND THE PREGNANEDIOL EXCRETION IN 15 DIABETIC PATIENTS WHO HAD LIVE BABIES.

Hospital Number	Case No.	Obstetric History	Weeks of pregnancy 28	29	30	31	32	33	34	35	36	37	38	39	40	type of delivery Remarks
99	1	2 alive	35.6		37.2		42.0	34.0	40.0	39.2	35.3					Caesarean section
666	2	1 stillborn					23.0	31.0	34.0	33.0	37.0					Spontaneous S.I.
57	3	2 stillborn				29.0	25.0	25.5	23.2	26.0	20.0	25.8				Toxaemia - Caesarean section
5573	4	-					14.7	19.3	17.6	28.6	25.0					Hypertension - Caesarean section
48822	5	-								34.2		32.0				Caesarean section
11885	6	-				35.5		34.4		40.0		34.4				" "
10463	7	1 stillborn					52.5	34.2		27.9	45.8					" "
9266	8	1 alive	34.0			36.0		44.0	35.0	33.0	28.4					" "
3123	9	-									47.6	52.0				Hypertension - S.I. Spontaneous
10142	10	4 alive												54.0	66.0	Spontaneous
12184	11	1 alive	31.0		28.6				43.0	55.0	50.0	37.0				" S.I.
10482	12	1 alive								62.4	77.6	34.2				" S.I.
12987	13	1 died			23.6		31.4		36.0		38.8	34.0				Caesarean section
12343	14	2 alive	35.8	42.0	39.0	36.4		45.0		40.4	34.0	33.6				" "
12349	15	"	34.0		37.8	37.2	35.0	38.6	32.7	38.2	40.0	35.4				" "

S.I. = Surgical Induction.

RESULTS.

The pregnanediol excretion in the live birth group is shown in Table 3 and Fig. 4. The excretion was within the normal pregnancy range except in cases 3 and 4. Case 3 was one of the cases of essential hypertension (180/110) mm.Hg.) and case 4 had toxaemia with albuminuria ($\frac{1}{2}$ to 2 gm./1000) and hypertension (150/100 mm.Hg.). Live babies which survived were delivered by Caesarean section in both these cases.

Table 4 and Fig. 5 show the pregnanediol excretion in 4 cases where intra-uterine foetal death occurred. In all cases the pregnanediol excretion level was maintained for 7 to 14 days after foetal death had occurred. Fig. 6 shows the pregnanediol excretion in one of these cases who had daily estimations made for 9 days after the foetal death and then every third day until delivery. The excretion was 32 mg./24 hours 16 days after the foetal death and there was a gradual fall during the next 3 weeks until a level of 15 mg./24 hours was found before delivery. The birth weights of the babies in this group varied from 6 to $7\frac{1}{2}$ lbs. although foetal

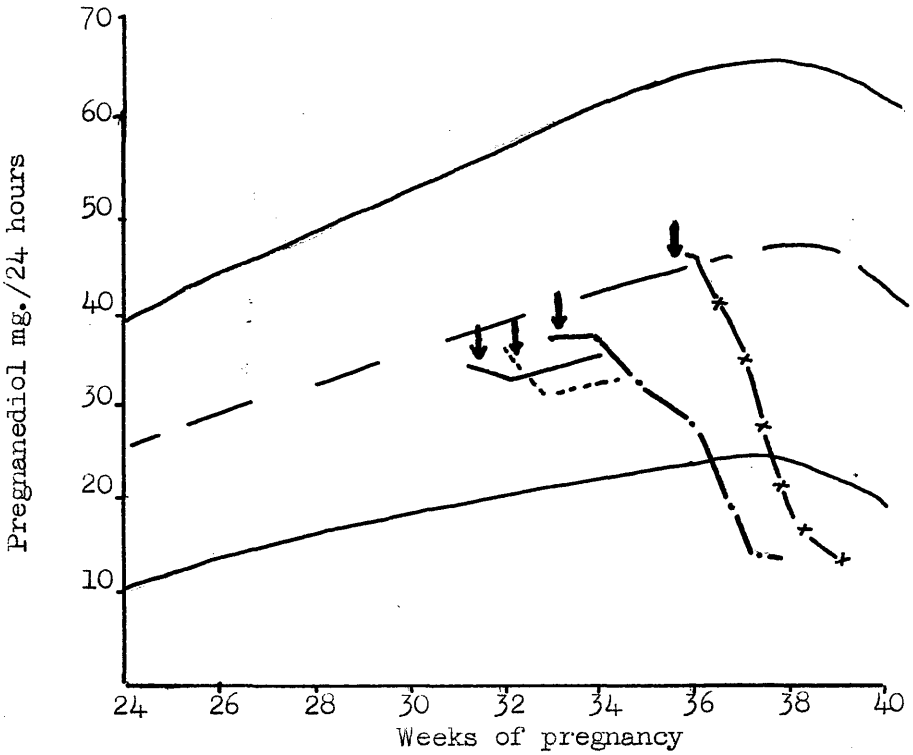


Fig. 5. THE PREGNANEDIOL EXCRETION IN 4 CASES OF FOETAL DEATH IN DIABETIC PREGNANCIES. (Table 4).

⬇ Indicates where the foetal death occurred.

——— Normal pregnancy range.

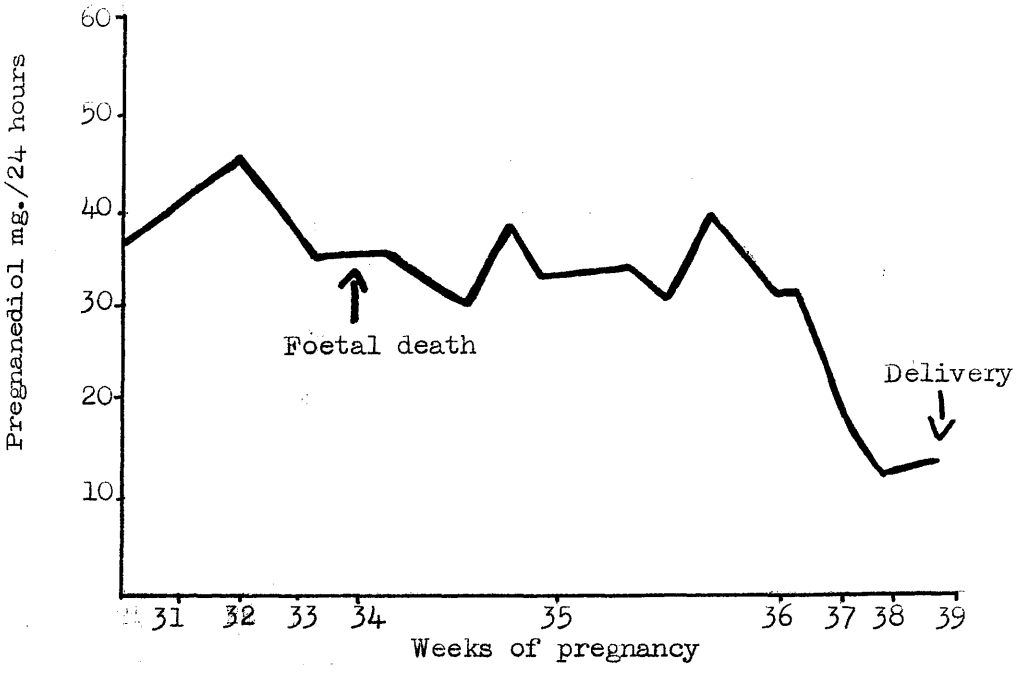


Fig. 6. Case 1. Table 4.

THE PREGNANEDIOL EXCRETION IN A CASE OF FOETAL DEATH FROM DIABETES.

Table 4. THE CLINICAL DETAILS AND THE PREGNANEDIOL EXCRETION IN 4 DIABETIC PREGNANT WOMEN WHO HAD STILLBIRTHS

Hospital Number	Case No.	Obstetric History	Weeks of pregnancy										Remarks
			31.	32	33	34	35	36	37	38	39	40	
7007	1				37.0	36.7 ↓	30.0	28.0	14.8	15.0			
9366	2	3 alive		36.0	31.0	↓32.2							
43527	3	2 alive						↓45.6	36.5	17.5	14.0		
10131	4	2 died	34.2	33.0	↓35.0	37.2							Hypertension

↓ Indicates where intra-uterine death occurred.

death occurred between the 33rd and the 35th week of pregnancy. The weights was thus increased relative to the duration of pregnancy.

DISCUSSION.

Nineteen diabetic pregnant patients had regular pregnanediol estimations made after the 28th week of pregnancy. Fifteen of these had live births and 4 had stillbirths.

The pregnanediol excretion in the group who delivered living babies was within the normal pregnancy range except in 2 cases, one complicated by hypertension and the other by toxaemia. It seems likely that the cause of the low pregnanediol in these cases was not the diabetes but the complication, namely the hypertension and the toxaemia.

The pregnanediol excretion in the 4 cases where intrauterine foetal death occurred was maintained at a normal level for a period of 7 to 14 days after the foetal death. These results suggest that the placenta continues to live after the foetus dies. They are in agreement with those of Rolland (1954), who, using

the method of Marrian, Sommerville & Kellar (1948) showed that the overall picture for the pregnanediol excretion in 44 diabetic pregnant patients, 12 of whom had stillbirths, differed little from that obtained in normal pregnancy. They are however at variance with those of Smith & Smith (1947) who, using the method of Venning (1938) showed that there was a low pregnanediol excretion in pregnancy diabetes. White (1949) used the findings of Smith & Smith (1947) as a basis for hormone therapy and showed an increase in the foetal survival when progesterone and oestrogen were administered in pregnancy diabetes. Peel (1955) was unable to show any significant increase in the foetal survival in diabetic patients treated with stilboestrol therapy. The results reported here indicate that progesterone production is normal in the diabetic pregnancy and consequently there is no rationale for replacement hormone therapy.

CONCLUSIONS.

The diabetic pregnant woman has a normal pregnanediol excretion.

Subnormal levels were found in two cases where live babies were born. One of these cases was complicated by hypertension and the other by toxaemia and thus the low values of pregnanediol excretion could have been due to the complications and not to the diabetes.

In 4 cases where intrauterine foetal death occurred the pregnanediol excretion was maintained at a normal level for 7 to 14 days after the foetus had died. It is postulated that placental insufficiency was not the cause of the foetal death in these cases.

It may thus be concluded from this small series that the pregnanediol assay is of no value in determining the outcome of the pregnancy in uncomplicated diabetes since a normal level of excretion can be maintained before and for sometime after intrauterine foetal death.

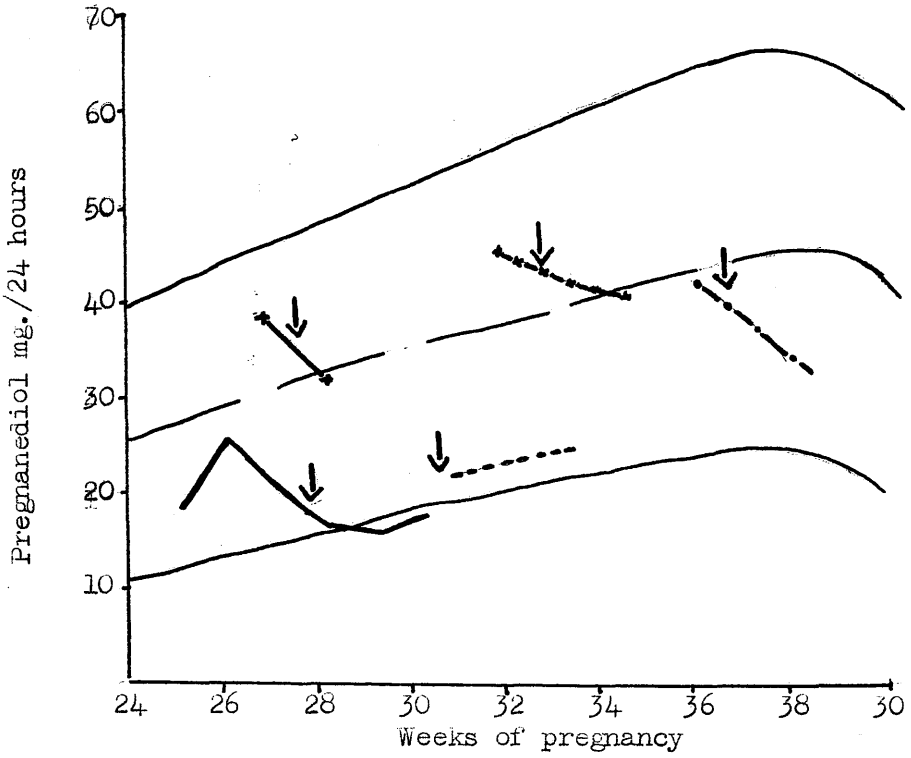


Fig. 7. THE PREGNANEDIOL EXCRETION IN 5 CASES OF FOETAL DEATH ASSOCIATED WITH RHESUS IMMUNISATION (Table 1).

↓ Indicates when the foetal death occurred.

_____ Normal pregnancy range.

Table 5. THE CLINICAL DETAILS AND THE PREGNANEDIOL EXCRETION IN 5 CASES OF FOETAL DEATH ASSOCIATED WITH RHESUS IMMUNISATION.

Hospital Number	Case No.	Birth Weight	Placental weight	Duration of pregnancy at foetal death (weeks)	Weeks of Pregnancy: 25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
12360	1	1 lb. 1oz.	6oz.	29	18.0	26.0		↓ 17.0	↓ 15.8	16.6										
2738	2	6 lb. 7oz.	4lb.	32								41.8	↓ 42.2	40.8						
109142	3	3 lb. 4oz.	1 lb. 4oz.	31						↓	22.2		24.1		↓	42.0	39.2	34.2		
9609	4	5 lb. 2oz.	1 lb. 6oz.	36																
1084	5	2 lb. 14oz.	1 lb.	28			38.0	↓ 33.0	34.0											
Average:		3 lb. 12oz.		31																

C. THE PREGNANEDIOL EXCRETION IN CASES OF FOETAL DEATH FROM RHESUS IMMUNISATION AND FROM CAUSES UNKNOWN.

It has been shown that in cases of foetal death in the diabetic patient normal levels of pregnanediol excretion may be found for 7 to 14 days after the foetal death. Cases of intra-uterine death from Rhesus immunisation and from unknown causes were next investigated.

The pregnanediol excretion in 5 cases of foetal death associated with Rhesus immunisation.

Pregnanediol estimations were made at regular intervals throughout pregnancy in 5 patients who were sensitised to the Rhesus antibody. Intra-uterine death occurred in all cases between the 27th and 36th week of pregnancy, the birth weights varying from 1 lb. 1oz. to 6lb. 7oz. with an average of 3lb. 12oz. which is considered normal for the average gestation period of 31 weeks.

The results of the pregnanediol estimations are shown on Figure 7 and Table 5. It will be noted that except in case 1, the excretion was within the

normal pregnancy range before and after the foetal death. In case 1 a significant drop (9 mg./24 hours) was noted before the foetal death and a level just below the lower limit of the normal pregnancy range was maintained for 19 days afterwards. This is the only case in which a subnormal birth weight was present (1 lb. 1oz. at the 28th week of pregnancy). In case 2 the placenta weighed 4lb., the pregnanediol excretion was within the normal pregnancy range but the baby, a hydrops, was heavy (6lb. 7oz.) for a 32 weeks' gestation.

It has been shown that in some cases of foetal death associated with Rhesus incompatibility, normal levels of pregnanediol excretion are found before and after the foetal death. The cause of the foetal death in Rhesus immunisation is often erythroblastosis, not placental insufficiency. Thus the placenta can continue to function and produce hormones after the foetus has died. The placental function can be demonstrated by the level of pregnanediol excretion.

TABLE 6. THE CLINICAL DETAILS AND THE PREGNANEDIOL EXCRETION IN 5 CASES OF FOETAL DEATH OF UNKNOWN AETIOLOGY.

Hospital Number	Case No.	Birth Weight	Duration of pregnancy at foetal death (weeks)	Weeks of pregnancy: 30	37	38	39	40
1638	1	1 lb. 8oz.	30	↓11.4				
12347	2	3 lb. 5oz.	37		↓17.0			
9816	3	7 lb. 3oz.	38			↓58.0		
5976	4	6 lb.	41				26.8 ↓	24.4
5103	5	7 lb. 12oz.	40			↓35.6	42.8	

↓ Indicates when the foetal death occurred.

The pregnanediol excretion in 5 cases of foetal death of unknown aetiology.

The clinical findings of this group are shown in Table 6. Of the 5 patients, 3 had foetal deaths between the 30th and 37th week of pregnancy and 2 between the 40th and 41st week. The cause of the foetal death was not known in any of these cases. In cases 1 and 2 the babies' weights were subnormal for the gestation period at the time of foetal death, but normal in the remainder.

The pregnanediol excretion is shown on Table 6. In cases 1 and 2, subnormal levels were found 2 and 4 days after the foetal death; in case 3, the level was within the normal pregnancy range 4 days after the foetal death. In cases 4 and 5, normal levels were found 2 and 4 days before and 1 and 2 days after the death of the foetus. The birth weights were subnormal in the cases where a subnormal excretion was found, and normal when the excretion was normal. Thus it may be assumed that in cases 1 and 2 placental insufficiency, judged by the subnormal weights of the babies and the low pregnanediol levels, was present,

and that if the foetal death in cases 3, 4 and 5 was caused by placental insufficiency, this was not demonstrated by the level of pregnanediol excretion or by the birth weights of the babies.

Summary and conclusions.

In 3 cases of foetal death of unknown aetiology and in 4 cases from Rhesus immunisation normal levels of pregnanediol excretion were found after the foetal death. The birth weights were normal for the gestation period except in one case associated with Rhesus immunisation where the baby, a hydrops, was 6lb. 7oz. at the 32nd week of pregnancy.

In 2 cases where the aetiology of the foetal death was uncertain, the pregnanediol excretion levels after the foetal death were subnormal for the gestation period and the birth weights were subnormal. Thus the cause of the foetal death was probably placental insufficiency. A fall in pregnanediol excretion to a subnormal level was found before the foetal death in one of the cases of Rhesus immunisation, but the baby's weight was subnormal.

It may be concluded that in some cases of foetal death of unknown aetiology or from Rhesus immunisation, the placenta can continue to function after the foetus has died and pregnanediol is excreted at a normal level. The cause of the foetal death in other cases may be placental insufficiency and in these cases subnormal or falling pregnanediol excretions are associated with subnormal baby weights.

D. THE PREGNANEDIOL EXCRETION IN CASES WHERE THE OBSTETRIC HISTORY WAS POOR.

Patients who have had an unexplained stillbirth or neonatal death are difficult to manage in a future pregnancy. There is always a possibility that the baby will again be stillborn but at present there is no test which will detect incipient "foetal distress". This investigation was undertaken to establish the value of the pregnanediol excretion in these cases, both to detect placental insufficiency and to determine when the baby should be delivered.

The patients in this group had a history of one or more stillbirths or neonatal deaths in a previous pregnancy. Details of the obstetric history and the method of delivery are given in all cases.

Case I. (Mrs. C.) age 30 years, had had a history of 3 abortions and a neonatal death of a baby weighing 5lbs. 9ozs. at the 36th week of pregnancy. In the next pregnancy she was admitted to hospital at the 27th week on account of premature

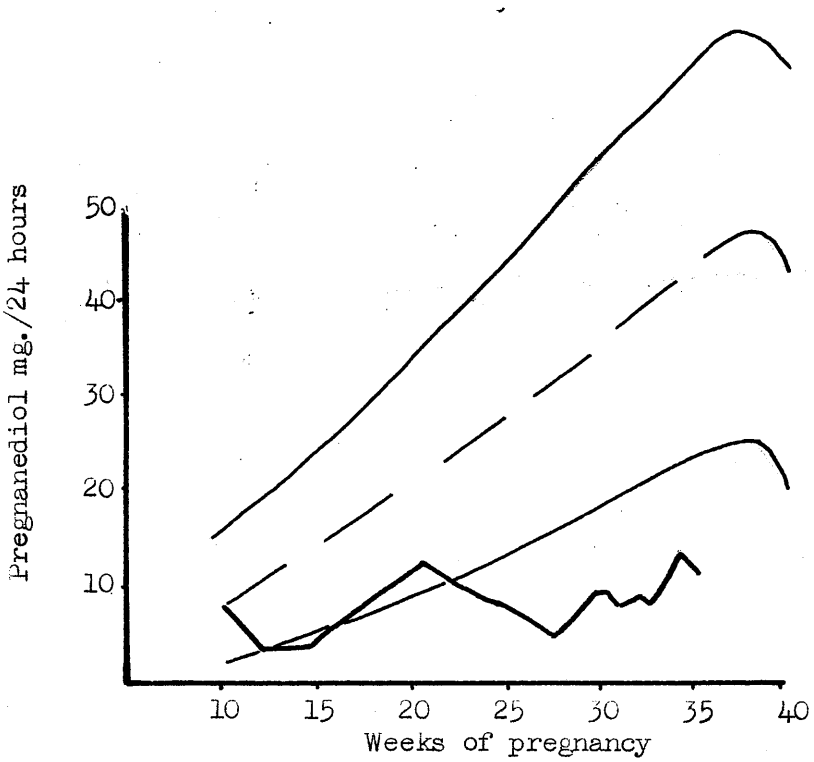


Fig. I. Case I. 6386/55. J.H.W.

1949 - Neonatal death $5\frac{9}{16}$ lb.

1951 - Abortion.

1952 - Abortion.

1954 - Abortion.

1955. Baby $3\frac{1}{16}$ lb. (alive)
Placenta $1\frac{3}{16}$ lb.
Spontaneous delivery.

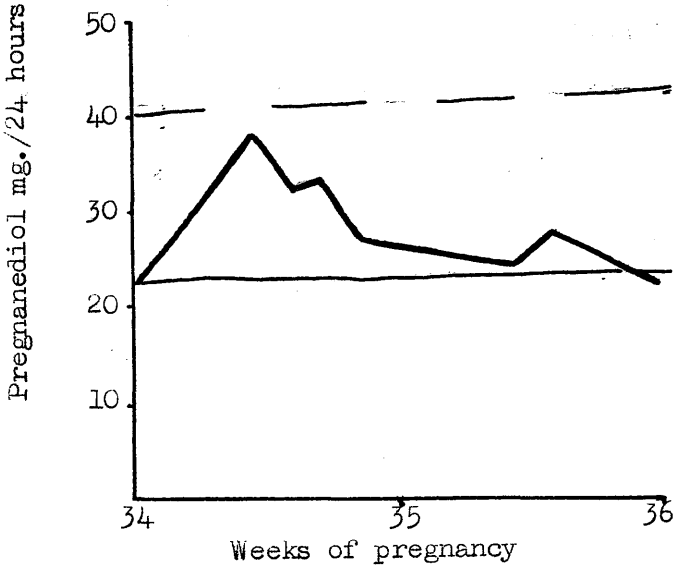


Fig. II. Case 2. Mrs. L. 1428/55 J.H.W.

1953 - Neonatal death. Full term 3lb.

1954 - Abortion

1955 - Caesarean section.
Baby 4lb. 14oz. (alive).
Placenta 1 lb. 2oz.

_____ Lower limit of the normal pregnancy range.

rupture of the membranes and at the 35th week she had a spontaneous vaginal delivery, the baby weighing 3lb. 1oz. The size of the baby was small for a pregnancy of 35 weeks maturity, but it survived. The placenta weighed 1 lb. 3oz. and was normal. The pregnanediol excretion is shown on Fig. 1. There was a fall below the normal pregnancy range at the 23rd week and then a low level was maintained until delivery. The correlation between the low birth weight and the low pregnanediol excretion is to be noted. Delivery in this type of case is often witheld until the foetus is approximately 5lb. in weight. Such a decision was not to be made as in this case spontaneous labour ensued. However it is suggested that the maturity rather than the size of the foetus should be taken into account in the decision to induce since the growth of the baby appears to be slow in cases where a low pregnanediol excretion is present.

Case II. (Mrs. L.), age 25 years, had a history of an abortion following a stillbirth weighing only 3 lb. at the 40th week of pregnancy.

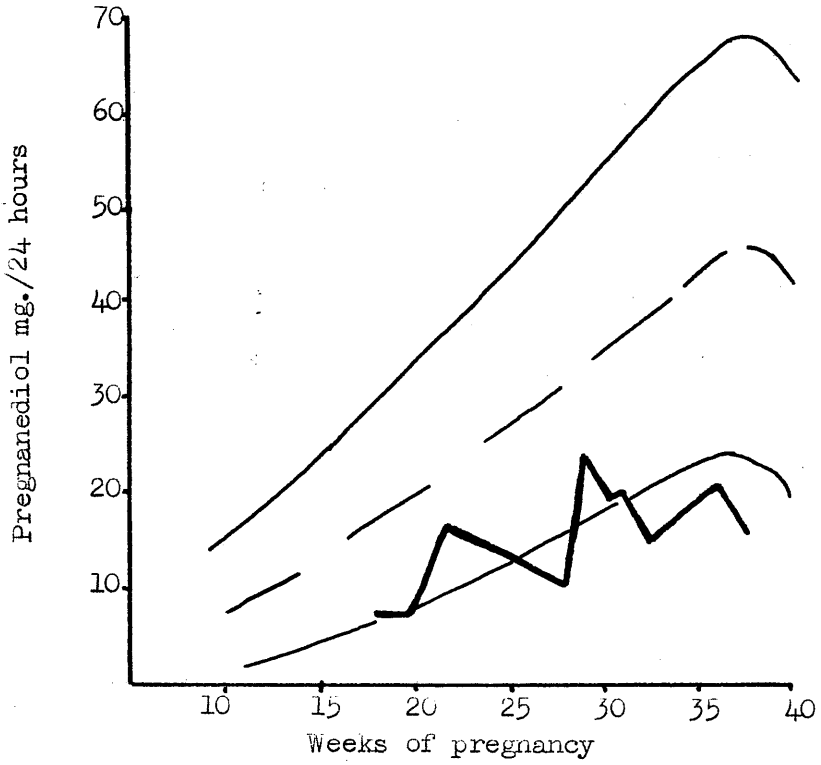


Fig. III. Case 3. Mrs. W. 7198/56. J.H.W.

1953 - Neonatal death 28 weeks

1954 - Neonatal death 28 weeks

1956 - Live birth 8lb. 8oz.

Placenta - 1 lb.

She remained well throughout the next pregnancy but the growth of the uterus was poor in comparison with the duration of amenorrhoea and Caesarean Section was performed at the 36th week. The baby weighed 4lbs. 14oz., which was small for a 36th week pregnancy but it survived. The placenta, 1 lb. 2oz. in weight, showed a few small areas of infarct and on histological examination signs of ageing and insufficiency was found. The pregnanediol excretion (Fig. II) was at the lower limit of the normal pregnancy range but there was a fall just before delivery. This is in agreement with the slow foetal growth and the placental histology and in association with the history could have been used as an indication for delivering this patient at the 36th week of pregnancy.

Case III. (Mrs. W.) had had stillbirths at the 28th week of her 2 previous pregnancies. In the next pregnancy the uterine size increased slowly and at the 37th week it was decided to induce labour surgically. A living 5lb. 8oz. baby, which survived, was delivered vaginally after a 6 hour labour. In this case the pregnanediol excretion (Fig. III) was maintained at

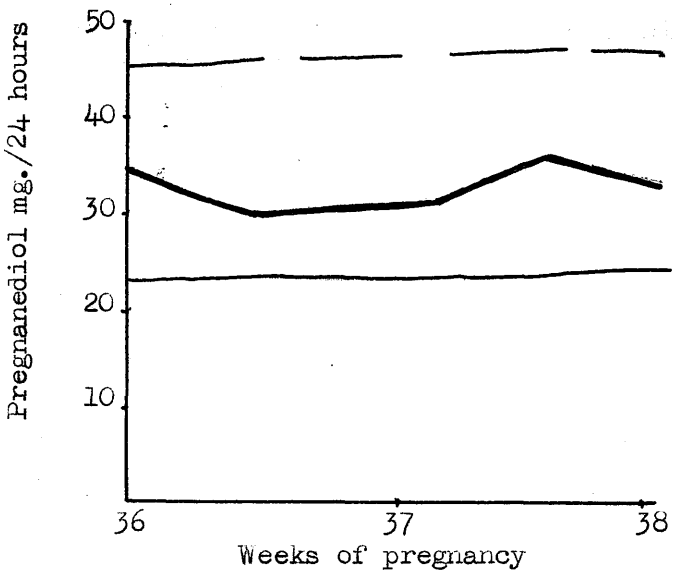


Fig. IV. Case 5. 33692/56. C.G.H.

1954 - Stillbirth 36 weeks
3lb. 3oz.

1955 - Stillbirth 34 weeks
3lb. 4oz.

1956 - Surgical induction 38 weeks.
Baby 6 lb. (alive)
Placenta 1 lb. 1oz.

a level below the lower limit of the normal pregnancy range from the 31st to the 37th week of pregnancy. The significance of this observation is uncertain as the baby was of reasonable weight and the placenta 1 lb. in weight was normal.

Case IV. (Mrs. H.) had a history of 2 intrauterine foetal deaths, one following an accidental haemorrhage. She was well throughout the next pregnancy and the baby continued to grow normally. She delivered a living 8lbs. 0½oz. baby at the 39th week. The duration of labour was 3 hours and the placenta appeared to be healthy and weighed 1 lb. 8ozs. The pregnanediol excretion at the 37th week was 58 mg./24 hours and at the 39th week it was 49 mg./24 hours. Thus the normal excretion levels in this case agreed with the good placental function and normal growth of the baby.

In her previous pregnancies, Case V., (Mrs. R.) had had 2 stillborn babies weighing 3lb. 3oz. and 3lbs. 4oz. and at the 36th and 34th weeks. In the next pregnancy the uterus continued to grow normally and labour was induced surgically at the 37th weeks.

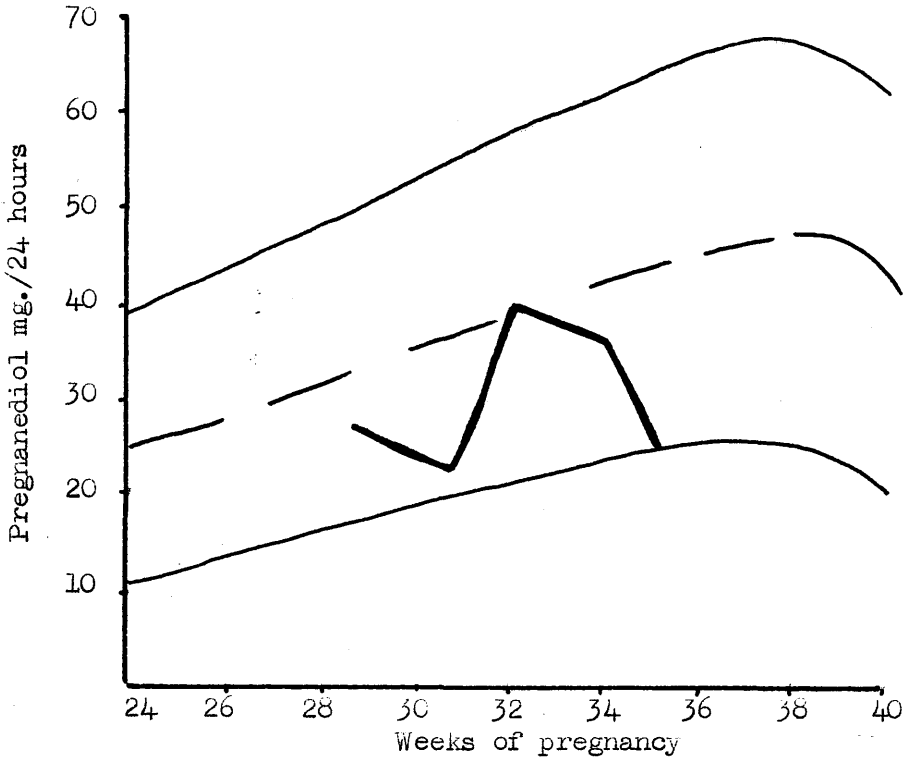


Fig. V. Case 6. 58189/56 C.G.H.

1944 - Neonatal death. Full term $4\frac{10}{16}$ lb.

1946 - Stillbirth 32 weeks.

1947 - Stillbirth 32 weeks.

1956 - Caesarean section.
Baby 5lb. 12oz. (alive)
Placenta 1 lb.

A living 6lb. baby was delivered and the placenta weighed 1 lb. 1oz. and was healthy. The pregnanediol excretion maintained at a normal level until delivery (Fig. IV) tallied with the successful outcome.

Case VI. (Mrs. A.) age 35 years, had been married 10 years and her 3 previous pregnancies had resulted in the delivery at the 40th week of a 4lb. 10oz. baby which died during labour, and 2 stillbirths at the 32nd week of pregnancy. Her next pregnancy was uncomplicated and a living 5lb. 12oz. baby was delivered by Caesarean section at the 35th week. The placenta weighed 1 lb. and on histological examination evidence of ageing and insufficiency was found. The pregnanediol excretion (Fig. V) was normal but started to fall at the 32nd week until at the 35th week it was below the lower limit of the normal pregnancy range. This fall could have been related to the senile changes which had occurred in the placenta and have been used as an indication for terminating the pregnancy.

Case VII. (Mrs. F.) had had one normal delivery followed by 3 stillbirths and an abortion. In her next pregnancy vaginal bleeding occurred at irregular

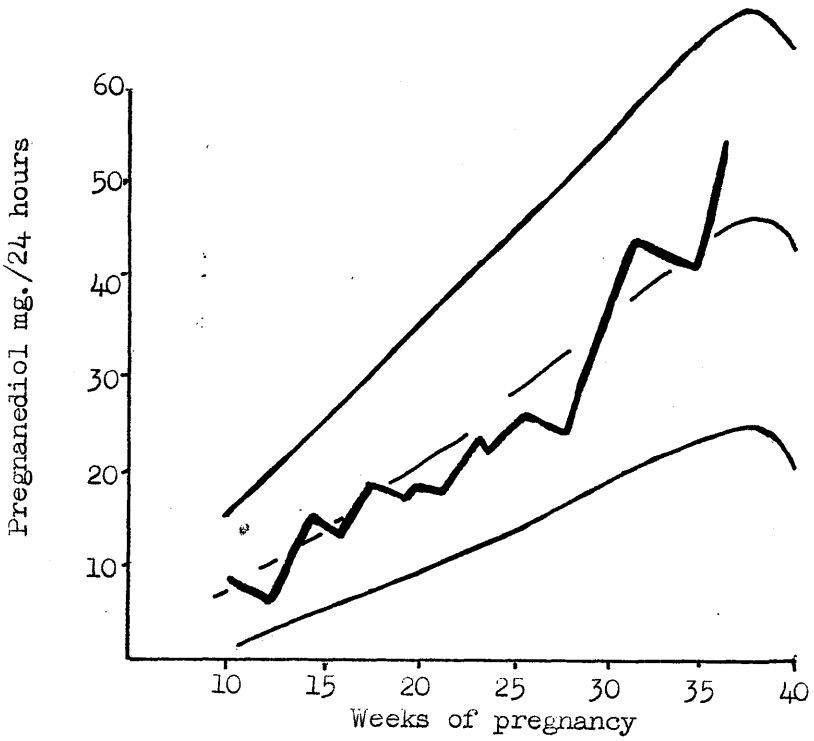


Fig. VI. Case 7. Mrs. F. 360/55. J.H.W.

1943 - Normal full term delivery
1947 - Stillbirth 28 weeks
1953 - Stillbirth 29 weeks
1954 - Stillbirth 32 weeks
1955 - Caesarean section
Baby 5lb. 4oz. (alive)
Placenta 1 lb.

intervals and she was kept in hospital from the 8th to the 36th week when the membranes ruptured spontaneously and the foetal heart became irregular. A living 5lb. 4oz. baby was delivered by Caesarean Section. The placenta weighed 1 lb. 4oz. and it appeared to be healthy on macroscopic and histological examination. Pregnanediol estimations (Fig. VI) made at regular intervals of 3 to 5 days throughout pregnancy, remained within the normal pregnancy range and reached a level of 54 mg./24 hours before delivery. In this case the pregnanediol excretion was in agreement with the placental histology. The cause of the continued vaginal bleeding may have been placental separation but it had no effect upon the pregnanediol excretion, the growth of the placenta nor of the baby. It is possible that the irregularity of the foetal heart was due to a cause other than placental insufficiency, since the pregnanediol excretion was high; the most likely cause was rapid engagement of the foetal head when the membranes ruptured in this multiparous patient.

Case VIII. (Mrs. B.) had a history of a neonatal

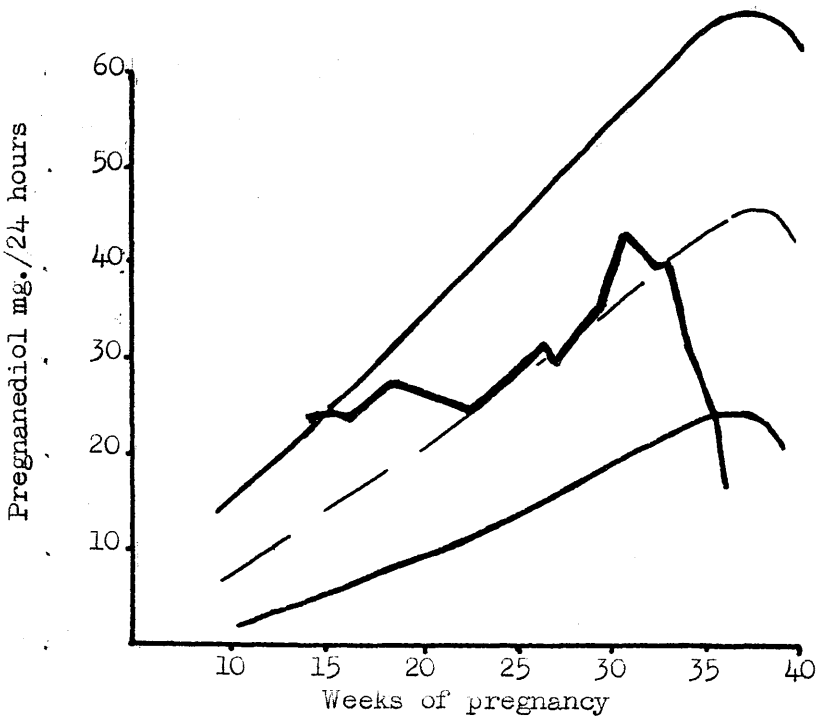


Fig. VII. Case 8. Mrs. B. 4755/55. J.H.W.

1952 - Neonatal death

1953 - Abortion

1954 - Abortion

1955 - Spontaneous delivery
(Foetal distress)

Baby 8lb. 10oz. (alive)

Placenta 1 lb. 14 oz.

death and 2 abortions in previous pregnancies. The next pregnancy was prolonged for 6 days after the expected date of delivery when labour occurred spontaneously and lasted 10 hours. There was "foetal distress" in the second stage of labour and the baby was asphyxiated at birth but it responded to resuscitation and survived. It weighed 8lb. 10oz. and the placenta weighed 1 lb. 14oz. Histologically the placenta showed evidence of poor function and postmaturity. The pregnanediol excretion (Fig.VI) was within the normal range till the 40th week when it dropped to 17 mg./24 hours. In view of the placental histology it seems probable that the cause of the foetal distress was placental insufficiency. It is reasonable to deduce that the fall in pregnanediol excretion was due to the reduced placental function and it is tempting to suggest that drop in excretion could have been used as the indication for immediate delivery in this case.

DISCUSSION.

The cause of the stillbirth and neonatal deaths in previous pregnancies in this group of patients was

not known but it is possible that placental insufficiency may have been present in some of the cases where small babies, born at term, did not survive. This complication could have recurred in subsequent pregnancies. It is difficult to assess the growth of the foetus clinically but it may be possible to demonstrate placental function by the pregnanediol assay.

A normal level of pregnanediol excretion was found in Cases IV, V, and VII who delivered live babies of average weight for the gestation period. In Case VII irregularity of the foetal heart was detected immediately the membranes ruptured but this may have been caused by the sudden engagement of the foetal head and not by placental insufficiency, the placental histology was in agreement with the pregnanediol excretion.

The fall in excretion noted in Case II, VI and VII was associated with placental senility detected on histological examination. Living babies were delivered by Caesarean section at the 35th week in Cases II and VI and spontaneously after a 10 hour labour in Case VIII, foetal distress was noted before

delivery in this case.

Cases I and III had low normal levels followed by a maintenance of excretion instead of a rise. The excretion in Case I remained at the low level of 14 mg./24 hours till delivery at the 35th week of pregnancy. The clinical association in this case was the low weight of the baby which was only 3lbs.loz. at the 35th week. The other foetus which was of low weight in relation to the maturity was Case II where a falling pregnanediol level was detected. A reasonable explanation cannot be found for the subnormal values detected in Case III and no clinical association was found.

CONCLUSIONS.

The results of the pregnanediol assay in this small series of cases where the obstetric history was poor lead to the following conclusions. Normal pregnanediol values suggest that the foetus is growing normally and that a successful outcome of the pregnancy can be anticipated.

Values which suddenly fall may indicate that

the placenta is showing signs of ageing and insufficiency and that delivery should not be postponed.

Consistently low values are of doubtful significance but in these cases the growth of the foetus may be slow and the maturity rather than the foetal size should be taken into account when the decision for induction of labour is weighed.

GENERAL SUMMARY AND CONCLUSIONS.

Some of the results presented in this section confirmed the opinions already expressed in the section on toxaemia of pregnancy in that a relationship was found between the subnormal levels of pregnanediol excretion and subnormal birth weights in cases of accidental antepartum haemorrhage, foetal death of unknown aetiology and in patients with a poor obstetric history. In some of these cases histological evidence of placental insufficiency was also found.

A normal level of pregnanediol excretion was found in 17 cases of uncomplicated diabetes and in 4 of these cases the level was maintained for 7 to 14 days after the foetal death had occurred. Similar results were obtained in 4 cases of intra-uterine death from Rhesus immunisation and 3 cases of foetal death of unknown aetiology.

Good correlation was found between pregnanediol excretion and the clinical findings in the group of patients who had a poor obstetric history and it was decided that the assay was of value in the

management of these cases.

It may be concluded that the pregnanediol assay may be of value in the management of some cases of accidental haemorrhage, diabetic pregnancy complicated by toxaemia and hypertension, and in cases where the obstetric history is poor.

When a small series of cases of foetal death from Rhesus immunisation and foetal death of unknown aetiology were investigated the results showed that placental function was maintained at a normal level before, and for some time after the foetal death had occurred.

FINAL SUMMARY AND CONCLUSIONS.

This work was undertaken to establish whether or not the urinary pregnanediol assay could be used as an indication of placental function in abnormal pregnancy.

A biochemical survey of two precipitation methods and of the chromatographic method of Klopper, Michie & Brown (1955) proved that the chromatographic method was the most accurate.

The blood "progestation activity" and the urinary pregnanediol excretion were compared in 10 normal women at the 34 to 36th week of normal pregnancy. No correlation was found between the two.

The daily variation on 3 consecutive days, in the pregnanediol excretion in these ten women, at the same period of pregnancy was found to be insignificant.

Regular estimations were made during pregnancy on 24 patients and the normal pregnancy range was established. A peak level of excretion was found between the 36th and 38th week, and there was a fall just before delivery in the majority of cases. The

excretion before and after delivery was studied in detail and only a slight fall in excretion was found on the day following delivery.

The range of excretion in early normal pregnancy is very wide, and for this reason the assay proved to be of limited value in the management of cases of recurrent and threatened abortion.

In 6 cases of recurrent abortion stilboestrol therapy neither increased nor decreased the pregnanediol excretion, and using the progesterone recovery test of Guterman (1953) it was impossible to foretell the outcome of a pregnancy in cases of threatened abortion.

Evidence was presented to show that the pregnanediol excretion may reflect placental function in cases of severe toxaemia. In many cases a relationship between the pregnanediol excretion, the birth weight and the placental histology was shown. Other factors which might be responsible for altering this relationship have been discussed.

In some cases of non-toxic accidental antepartum haemorrhage the same relationship between the

pregnanediol excretion, the birth weight and the placental histology was noted.

A small series of cases of diabetes in pregnancy was investigated, and the pregnanediol excretion in uncomplicated diabetes was found to be normal. In cases of intra-uterine death, normal levels were found in diabetes, Rhesus immunisation and in foetal death of unknown aetiology.

It may be concluded that the urinary pregnanediol assay can be used to indicate placental function in some abnormal conditions of late pregnancy. The results obtained may give considerable assistance to the clinician, in assessing cases where a reduction in the functioning capacity of the placenta is suspected.

ACKNOWLEDGEMENTS.

I have pleasure in acknowledging my indebtedness to the Consultant Staff of the Jessop Hospital, in particular Mr. Patrick and Professor C.S.Russell for allowing me to investigate their patients and for their advice and interest in this work; to Dr. C.G. Paine for his guidance and help at all stages of the investigation and for permission to use the histological reports on the placentae and on the Hooker Forbes technique. I am indebted to Dr. F.L. Mitchell for advice, assistance and encouragement in the biochemical part of this investigation; to Dr. A. Klopper of the Medical Research Council Unit, Edinburgh for giving me details of his method before publication and for help and encouragement; to Mr. R. Hall, F.I.M.L.T. who kindly carried out many of the duplicate estimations of pregnanediol throughout, and to Miss M. Early, B.Sc., for technical assistance. I am grateful to the medical, nursing and labotatory staff of the hospital for assistance in many ways, to Dr. P.M. Smith for encouragement and advice and to my sister Dr. A.C. Coyle for translating the German literature.

My grateful acknowledgements are also due to the Board of Governors of the United Sheffield Hospitals for generously financing the work.

PUBLICATIONS.

Part of the work presented in this thesis has been previously published, the references to these publications being:-

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