THE EXCRETION OF CHORIONIC GONADOTROPHIN BY PREGNANT DIABETICS

BY

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The quantitative estimation of chorionic gonadotrophin (C.G.) in the blood and urine of pregnant women has assumed new importance from the diagnostic point of view since the work of White (1937, 1943, 1946, 1947), White *et al.*, (1939), and White and Hunt (1943) on pregnancy complicated by diabetes and of Smith and Smith (1935, 1936, 1941, 1948), Watts and Adair (1943), and Smith, Smith, and Hurwitz (1944) on the toxæmias of pregnancy.

Although the introduction of insulin greatly reduced the maternal mortality rate in pregnant diabetics (Kramer, 1935; Lawrence and Oakley, 1942), the foetal mortality in such cases has not shown a corresponding decline (Herrick and Tillman, 1938; Gilbert and Dunlop, 1949; Barns and Morgans, 1949). It is evident that foetal wastage remains the great obstetrical problem in diabetes, and in some series of cases the foetal loss rate has been over 50%.

Set in this background, therefore, the great reduction in foetal mortality rate obtained by White (1946, 1947), using hormonal therapy in the form of oestrogens and progesterone in a proportion of these cases, is very striking indeed. Estimations were made by White of the serum chorionic gonadotrophin, using corpora lutea in immature female rats as the end-point of the biological assay: urinary pregnanediol was estimated by the Venning (1937) method and subsequently by that of Astwood and Jones (1941). A few assays of oestrogens in the serum were performed by biological methods.

From a study of 300 consecutive cases of pregnancy complicated by diabetes White (1947) found that such patients could be divided into two groups, each carrying an entirely different prognosis.

The first group consisted of 66 patients in whom the C.G. readings throughout pregnancy were classified as normal. In this group the foetal survival rate was 97%, the incidence of premature deliveries was 0, and that of pre-eclampsia 2%. The second group (over 75% of the 300 consecutive cases) was said to suffer from "hormonal imbalance" during pregnancy. The C.G. levels were abnormally high, and this finding was often associated with a low urinary pregnanediol and a low serum oestrogen. Of the patients in this group, 58 received no hormonal therapy and the foetal survival rate was only 47%; premature delivery occurred in 40% and pre-eclampsia in 50%. In addition many of these patients delivered babies which were grossly over weight.

Since this change in C.G. level preceded, often by some weeks, any obstetrical accident it was concluded that a rise in the serum C.G. could be regarded as the harbinger of an impending disaster. A group of 174 cases showing "hormonal imbalance" were treated with oestrogen and in most cases with progesterone as well. In these patients the foetal survival rate rose to 90%; premature delivery occurred in only 5%, pre-eclampsia in 5%, and the majority of patients receiving this treatment delivered babies of normal size.

A method of assay of C.G. depending on the increase in prostatic weight in immature rats and based on the use

of the International Standard preparation of C.G. is described elsewhere (Loraine, 1949). This method was applied to the estimation of C.G. in the urine of normally pregnant women. In these normal cases very high readings, such as 25,000–30,000 i.u. of C.G. per 24 hours, were obtained from the 40th to the 80th day of pregnancy as calculated from the date of the last menstrual period. This was followed by a gradual decline, and from the 100th day until the end of pregnancy the excretion of C.G. was between 4,000 and 10,000 i.u. per 24 hours.

In Fig. 1 is shown the complete range of all the estimations made on the urines of normal pregnant women. The figures are quantitatively similar to those of Jones, Delfs, and Stran

(1944), who also used the International Standard but estimated C.G. in the serum. Curves of the same general shape have been obtained by other workers, who expressed their results in

arbitrary "rat units" (Cole and Saunders, 1935; Browne and Venning, 1936; Smith and Smith, 1936; Boycott and Rowlands, 1938; Browne, Henry, and Venning, 1939; Siegler, 1939; Rakoff, 1939; Taylor and Scadron, 1939; Venning, 1948). Although the series of cases was a relatively small one, the variations in the amount of C.G. excreted per 24 hours during the second and third trimesters of pregnancy were smaller than has been generally supposed, and much of the apparent variation shown by previous workers has probably been due to the use of relatively inaccurate assay methods.

The number of pregnant diabetics studied is as yet too small to assess adequately the effect of stilboestrol in reducing the foetal mortality rate. The present communication is concerned with the excretion of C.G. by diabetic women and the effect of oestrogens on this excretion in normal women and in diabetic women.

Methods.—The method used depends upon the increase in the prostatic weight in immature rats after subcutaneous administration of the urine to the animals. The results of the assay are always expressed in i.u. of C.G. per 24-hour specimen of urine. The technique is described in detail in the previous paper.

Results

The effect of orally administered stilboestrol on the C.G. excretion in two normally pregnant women was investigated. Fourteen pregnant diabetics were considered in all, and 11 of these had C.G. estimations made at approximately weekly intervals after diagnosis. Only a few readings were made on the other three patients. Stilboestrol was administered to these diabetics, who after 100 days of gestation consistently showed an excretion of C.G. above the normal level of 10,000 i.u. per 24 hours. Six of the 14 cases were shown to have abnormally high C.G. readings at some stage of their pregnancy, and the findings in these cases are discussed below. In the other eight patients the excretion of C.G. appeared to be normal.

FIG. 1.—Total range of 136 estimates of the excretion of chorionic gonadotrophin in normal pregnancy.

Normal Pregnancy

Case 1.—After two weeks' control period stilboestrol in dosage of 25 mg. a day was started at the 21st week of pregnancy (Fig. 2). The dose was increased to 30 mg. a day at

FIG. 2.—Excretion of chorionic gonadotrophin by normal and diabetic women treated with stilboestrol. The small figures denote doses (mg. per day) and are placed at the times these were started. (Cases 1-4).

the 23rd week. A sharp fall in the C.G. excretion occurred, the reading climbing back to just below its original level on cessation of the treatment.

Case 2.—After one week's control period stilboestrol in dosage of 30 mg. a day was given at the 34th week of pregnancy (Fig. 2). A modest depression of the C.G. level ensued initially, but when the same dose of stilboestrol was continued the excretion of the hormone returned to its original level, this occurring after approximately three weeks' therapy.

Pregnancy in Diabetics

Case 3.—Estimations made from the 17th to 23rd weeks of pregnancy showed an abnormally high excretion of C.G. (Fig. 2). Stilboestrol in relatively small dosage was started at the 23rd week and it produced a significant fall, but the reading rose to slightly above its original level on cessation of the therapy. Subsequent therapy with larger dosage started at the 28th week produced another dramatic fall in the C.G. excretion, but an "escape" phenomenon occurred when the same dosage was maintained. This patient was delivered at the 36th week by caesarean section. The weight of the baby was 6 lb. 7 oz. (2.92 kg.) and of the placenta 1 lb. 10 oz. (0.74 kg.).

Case 4.—An abnormally high C.G. excretion was found when the first estimations were made on this patient at the 27th week of pregnancy (Fig. 2). The dosage of stilboestrol started at the 31st week was rapidly increased by 5 mg. a day to 30 mg. a day and maintained at this level. A rapid initial fall in the C.G. excretion was observed, but this was followed by a rise to approximately the original level after four weeks' treatment. The patient was delivered vaginally at 36 weeks. The birth weight of the foetus was 4 lb. 11 oz. (2.13 kg.) and of the placenta 1 lb. 3 oz. (0.54 kg.).

Case 5.—The very high readings of C.G. obtained in early pregnancy in this patient were originally thought to indicate a delayed peak period, but the excretion remained consistently high well after the 100th day of pregnancy (Fig. 3). Stilboestrol in dosage of 25 mg. a day was started at the 15th week of pregnancy, and this was increased to 30 mg. a day at the 17th week. A fall in the excretion of C.G. occurred, but the titre rose again on cessation of the treatment. Therapy was restarted at the 20th week in dosage of 20 mg. a day, and this was increased to 30 mg. a day at the 25th week. This resulted in a fall in the urinary excretion of C.G. to 10,000 i.u. by the 27th week. After that, however, while the therapy continued the readings climbed steeply. Apparently the patient had

become quite refractory to stilboestrol in the dosage given. The oestrogen was stopped at the 30th week of pregnancy and restarted at the 31st week. Some degree of sensitivity appeared to have been re-established as a slight fall in the

FIG. 3.—C.G. excretion in pregnant diabetics. The small figures denote doses (mg. per day) and are placed at the times these were started. (Cases 5 and 6).

excretion was obtained with a smaller dosage of stilboestrol. This patient was delivered by caesarean section at the 37th week. The weight of the baby was 6 lb. 1½ oz. (2.76 kg.) and of the placenta 1 lb. 2 oz. (0.51 kg.).

Case 6.—The rise in C.G. excretion in this patient occurred at the 24th week of pregnancy (Fig. 3), readings before that time being comparatively normal. Stilboestrol in dosage of 20 mg. a day was started at the 29th week. A transitory depression of the reading was obtained, but again the "escape" phenomenon was observed. A dose of 300 mg. given to induce labour approximately halved the C.G. reading. This patient was delivered by caesarean section at 41 weeks. The foetus weighed 9 lb. 4 oz. (4.2 kg.) and the placenta 1 lb. 15 oz. (0.88 kg.).

Case 7.—The excretion of C.G. was relatively normal in this patient until the 28th week of pregnancy, when a steep rise occurred (Fig. 4). Stilboestrol in dosage of 20 mg. a day was

FIG. 4.—C.G. excretion in pregnant diabetics. The small figures denote doses (mg. per day) and are placed at the times these were started. (Cases 7 and 8).

started at the 31st week and produced a dramatic fall in the titre, but the reading climbed rapidly again while the therapy continued. This patient developed a mild degree of pre-eclampsia at 32 weeks. Delivery was by caesarean section at 34 weeks. The baby weighed 6 lb. 3 oz. (2.81 kg.) and the placenta 1 lb. 5 oz. (0.6 kg.).

Case 8.—Stilboestrol was administered to this patient at the 21st week of pregnancy for experimental purposes although the C.G. excretion was not unduly high (Fig. 4). A characteristic depression of the titre occurred, but the excretion rose when the treatment was stopped. No further readings were made until considerably later in pregnancy, when, although the patient was receiving massive doses of oestrogens (up to 120 mg. a day of stilboestrol supplemented by 100 mg. a day of oestradiol

benzoate at the 34th week), the C.G. level remained very high, again indicating refractoriness to the oestrogen. Delivery was by caesarean section at the 36th week of pregnancy. The foetus weighed 9 lb. 15½ oz. (4.53 kg.) at birth and the placenta 2 lb. (0.9 kg.).

In Fig. 5 the results are shown in those pregnant diabetics in whom the excretion of C.G. was within normal limits.

FIG. 5.—C.G. excretion in pregnant diabetics without hormonal therapy.

As in the previous series of normal cases, there is relatively little variation.

Discussion

From the evidence cited above it is apparent that a proportion of pregnant diabetic women (6 out of 14 studied) showed an abnormally high excretion of C.G. as compared with normally pregnant women. In the remaining eight diabetics the readings were perfectly normal (Fig. 5). Hormonal therapy was given only to those patients who after 100 days of gestation consistently showed a C.G. excretion of over 10,000 i.u. in 24 hours.

Stilboestrol when administered orally to normal and diabetic women, even in relatively small dosage, invariably produced a fall in the C.G. excretion. The degree of depression varied from case to case but tended to be more pronounced in early than in late pregnancy. On a constant dosage of the oestrogen, however, this depression was not maintained, the readings tending to climb back to their original level while the patient was still receiving treatment. This may be described as an "escape" phenomenon. At this stage an increase in dosage of stilboestrol temporarily produced another depression of the reading. Certain patients, however, seemed to become completely refractory to stilboestrol even in massive dosage. This is well illustrated by Case 8, which in the later stages of pregnancy was receiving 120 mg. of stilboestrol a day supplemented by oestradiol benzoate without depression of the C.G. level. There is some evidence (see Cases 3 and 5) that by cessation of the treatment for a few days sensitivity to the drug may be re-established and a depression of the C.G. level produced by a dose smaller than that previously employed.

The effect of stilboestrol on the C.G. excretion can therefore be considered to have five phases: (1) initial depression, (2) subsequent escape, (3) further depression by increase in dosage, (4) refractoriness after prolonged treatment, and (5) increased sensitivity to oestrogen on restarting treatment after stopping it for a few days.

The mechanism of action of oestrogens in producing these effects is not clear. Smith and Smith (1948), who first observed the rise in C.G., considered it a "compensatory" one due to lack of oestrogen and progesterone, and showed that oestrogen therapy will correct this.

Studies in the experimental animal have shown that oestrogen may suppress or inhibit the gonadotrophic activity of the anterior pituitary (Meyer and Biddulph, 1941; Meyer, Biddulph, and Finnerty, 1946). In the human, Jones and MacGregor (1936) observed that the

injection of oestrogen into women at the menopause resulted in a cessation of the increased gonadotrophic hormone excretion in the urine, and Albright (personal communication to Professor J. H. Gaddum, September, 1948) obtained a depression of the follicle-stimulating hormone titres in the urine of menopausal women by the oral administration of stilboestrol. The rationale of oestrogen therapy in Cushing's syndrome (Dunn, 1938; Rakoff, Cantarow, and Paschkis, 1941) and in acromegaly (Schrire and Sharpey-Schafer, 1938; Goldberg and Lissner, 1942) is in virtue of the inhibitory effect produced on the anterior lobe of the pituitary. There does not appear, however, to be any previously published work on the effect of oestrogens in depressing the excretion of the gonadotrophic hormone elaborated by the placenta during pregnancy.

In the pregnant diabetics studied so far, no definite correlation has been found between the C.G. excretion on the one hand and the weight of the baby and placenta on the other. There also appeared to be no relation between the C.G. excretion and the insulin requirements during pregnancy in these patients.

Summary

Estimates have been made of chorionic gonadotrophin (C.G.) in the urine of 14 pregnant diabetics. The prostatic weight in immature rats was the end-point of the assay.

In 6 of these 14 cases the figures for C.G. excretion obtained were abnormally high when compared with that of normal women.

The administration of oestrogens caused an evanescent fall in the amount of C.G. excreted in the urine per 24 hours.

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VITAMIN A, TOTAL CAROTENOIDS, AND THYMOL TURBIDITY LEVELS IN PLASMA

TEST IN NORMAL SUBJECTS RESIDING IN THE
MIDLANDS DURING 1947

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In the course of a previous investigation into the cause of nystagmus in coal-miners we estimated the levels of vitamin A and total carotenoids in the plasma (Campbell and Tonks, 1948). Thymol turbidity tests were performed at the same time on a number of cases in order to exclude the possibility of liver dysfunction as a cause of fluctuation in these constituents. Since the diet of the majority of people had been rather drastically altered by war and post-war conditions in Great Britain, we were obliged to establish "normal" figures for comparison. It was thought that the values obtained might be of practical interest to other investigators engaged on similar problems.

Methods

Estimation of Vitamin A and Total Carotenoids in Plasma

Clausen's application of the Carr-Price colorimeter method was the means used in this procedure. The colour was measured in a Hilger absorptiometer, using filters specially designed for these estimations. The results were read off from standard curves prepared as suggested by Yudkin (1941).

The following reagents were used: (1) ethyl alcohol (redistilled); (2) petroleum ether (boiling point 40–60° C.); (3) chloroform—alcohol-free and dried over anhydrous sodium sulphate; (4) antimony trichloride solution 30% (w/v) in dry chloroform; this is a saturated solution freshly prepared and filtered, and is cleared of any turbidity by the addition of one drop of acetic anhydride.

Preparation of Standard Curves.—Carotenoids:—For this purpose pure β -carotene was obtained from the National Institute for Medical Research. A weighed amount (about 0.25 mg.) was dissolved in 100 ml. of petroleum ether (boiling point 40–60° C.). Aliquots of this solution were made up to 5 ml. with petroleum ether, and readings were taken in the absorptiometer to determine the standard curve for β -carotene, using the special filter combination provided by Hilger for carotenoids. **Vitamin A:**—Capsules of vitamin A alcohol of high purity were very kindly given to us by Roche Products, Ltd., to whom we offer our thanks. The contents of one capsule were weighed and

made up to a known volume with chloroform. Under our conditions 1 ml. of this solution contained 27.25 i.u. Each aliquot taken was made up to 0.5 ml. with chloroform, and then 5 ml. of antimony trichloride solution was added. The resulting colour was read off on the absorptiometer within 10 seconds of the addition of the reagent, using the special combination of filters designed by Hilger for the estimation of vitamin A.

Correction Curve for the Blue Colour Produced by β -carotene and Antimony Trichloride.—A solution of a weighed quantity of β -carotene (1.2 mg.) was made up to 100 ml. with chloroform. Aliquots were made up to 0.5 ml., and 5 ml. of antimony trichloride solution was added. The special filter for vitamin A was used in the absorptiometer, and readings were taken within 10 seconds of mixing the reagents.

From these readings graphs were constructed so that results could be ascertained with accuracy and speed.

Two millilitres of plasma was placed in a stoppered centrifuge tube, 2 ml. of ethyl alcohol was added slowly, and the contents thoroughly mixed. To this mixture was added 4 ml. of petroleum ether. The stopper was inserted and the tube shaken at frequent intervals for half an hour. The mixture was then allowed to settle into its two layers or was centrifuged. An aliquot of the top layer (usually 3.6 ml.) was then transferred to an absorption cell, made up to 5 ml. with petroleum ether, and a reading taken using the carotenoid filter. The equivalent value in terms of β -carotene was then obtained from the standard curves and calculated for 100 ml. of plasma.

The petroleum ether solution in the cell was evaporated to dryness by placing the cell in a bath of warm water (40–60° C.). A gentle current of dry air or nitrogen was used to assist evaporation. The residue was dissolved in 0.5 ml. of dry chloroform. With the use of the special vitamin-A filter the cell was placed in the rack of the absorptiometer and 5 ml. of antimony trichloride solution added. The reading was taken within 10 seconds of the addition of the reagent.

The amount of vitamin A present in the cell was calculated by reading the value from the standard curve for vitamin A and subtracting the amount of blue colour due to the reaction of the antimony trichloride with the estimated amount of carotenoids present. From this value the amount in 100 ml. of plasma was calculated.

Thymol Turbidity Test

This test was originally described by MacLagan (1944), but in order to get more precise comparable values the modification of Ley, Lewis, and Davidson (1946) was used, in which a barium sulphate suspension was employed as a standard and the turbidity was measured in the Hilger absorptiometer. The results given in this paper, therefore, are expressed as being equivalent to the corresponding amount in millilitres of barium sulphate suspension.

A slight modification was introduced—namely, the performance of a blank test with 0.1 ml. of plasma and 6 ml. of 0.9% sodium chloride. The reading of this blank test on the standard barium sulphate suspension curve was subtracted from the value obtained in the usual way in the case of lipaemic or slightly haemolysed plasma.

Results

Vitamin A and Total Carotenoids

Our series consisted of 133 normal adults (116 men and 17 women) varying in age from 18 to 73 years. They included housewives, office workers, factory hands of all types, and labourers, and came from all walks of life. They were tested only when free from infection, as Brenner and Roberts (1943) found that feverish colds reduced the blood level of vitamin A by one-half. All specimens of blood were collected at approximately three hours after