EXCRETION OF CORTICOSTEROIDS BY INFANTS OF DIABETIC AND PRE-DIABETIC MOTHERS*

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Infants born to diabetic women are usually larger and heavier than average for their gestational age and often bear a superficial resemblance to cases of Cushing's syndrome (Åkerrén, 1954; Jackson, 1955; Farquhar, 1956a; Roszkowski and Janczewska, 1964).

On this account, various workers have investigated the excretion of adrenal steroids by these infants but the results have been inconclusive, possibly due to the fact that methods for the assay of groups of adrenocortical steroids have been used—methods primarily designed to measure the principal steroid metabolites excreted by adults and of limited application to studies of newborn infants with their unique steroid metabolism (Cathro, Birchall, Mitchell, and Forsyth, 1963). There is a further drawback to the use of these methods in analyses of urine obtained from newborn infants, in that accurate correction to exclude the contribution of the large quantities of non-specific chromogens that are characteristic of this age-group is somewhat difficult (Birchall and Mitchell, 1965).

Using such methods for the assay of groups of steroids, Rose (1960) found the corticosteroid excretion of infants of diabetic mothers to be normal, while Björklund (1954) and Farquhar (1956a) found it increased, and Field, Smith, and Reardon (1963) reported that the output of Porter-Silber chromogens in the urine of such infants when they developed respiratory distress was approximately twice as high as the output of these compounds in stressed premature infants born to women who did not suffer from diabetes.

Analysis of amniotic fluid has been no more rewarding. Hoet (1954) found appreciable quantities of corticoids in the amniotic fluid obtained from a pregnant diabetic woman, whereas the same techniques could not demonstrate the presence of corticosteroids in the amniotic fluid from normal controls. In contrast, Baird and Bush (1960) found no significant differences between the amniotic fluids obtained from normal pregnant women and from diabetics, when they estimated the levels of cortisol and cortisone.

Evidence from anatomical studies carried out on infants of diabetic women has also been inconclusive with regard to the degree of development of adrenal tissue. Miller and his colleagues (Miller, Johnson, and Durlacher, 1944; Miller, 1945) reported that occasionally the adrenal glands of such infants might be increased in size and weight, whereas this was not found in a large series of necropsies on infants of diabetic mothers reported by Driscoll, Benirschke, and Curtis (1960).

These inconsistent findings led us to investigate the individual neutral steroids excreted in the urine of infants born to diabetic mothers, since this approach, though technically more difficult, was considered preferable to the use of the group methods of steroid assay with their doubtful or, at best, limited, applicability at this period of life.

Materials and Method

Urine was collected on days 1, 2, 3, and 6, from 8 infants born to women with diabetes mellitus and from 6 infants born to women with pre-diabetes. The average gestational age at the time of birth of the infants of diabetic mothers was 36·3 weeks (range 35-37·5 weeks) while the average gestational age of the infants born to pre-diabetic mothers was 37·3 weeks (range 36-38 weeks). Mothers were classified as diabetic if they required treatment with insulin or with drugs following the puerperium, and as pre-diabetic if drugs or insulin were unnecessary following the birth of the baby. In some cases, the diabetogenic tendency was so mild that only dietary measures were required during the pregnancy, but in all of these, the diagnosis of diminished tolerance to carbohydrate was confirmed by means of glucose tolerance tests.
The control specimens of urine were obtained on days 1, 2, 3, and 6 from 14 healthy infants born uneventfully at term to mothers who had no endocrine abnormality. Specimens were also obtained from 10 full-term infants who were stressed at birth or in the neonatal period. Most of these suffered severe asphyxia at birth but 3 had haemolytic disease requiring exchange transfusion. A further 16 infants studied have been classified as premature on the basis of a birth weight of 2·5 kg. or less. Any 24-hour urine samples considered to be incomplete were discarded. In all but two instances the infants studied were male. Complete 24-hour specimens of urine were collected from the mothers during the period immediately following delivery.

Individual neutral steroids in the urine samples from infants were assayed by the method of Birchall, Cathro, Forsyth, and Mitchell (1963). In this method, an extract of the unconjugated steroids originally present in the urine and of steroids released by a two-stage enzyme hydrolysis, which will cleave steroid sulphates as well as steroid glucosiduronates, is subjected to preliminary purification and to chromatography, which allows subdivision of the steroids into three fractions on the basis of polarity. Fraction I contains 11-deoxy-17-oxosteroids and other relatively non-polar compounds, Fraction II contains the 11-oxy-17-oxosteroids, and Fraction III the corticosteroids. Final chromatography of the steroids in each fraction is followed by staining techniques which permit direct measurement on paper chromatograms. Corrections for losses of steroid during the estimations are applied on the basis of the recovery values for 4·3C steroids added to the urine samples at the outset.

The specimens from the mothers were assayed for 17-hydroxycorticosteroids by the method of Appleby, Gibson, Norymberski, and Stubbs (1955). 17-oxosteroids were assayed by a modification of the method of Norymberski, Stubbs, and West (1953).

The number of infants of diabetic mothers studied was unavoidably small, since the region served by the obstetric units has a limited population, and since attempts at accurate collection of 24-hour urine specimens from female infants were found impracticable. For purposes of comparison it is fortunate that all the infants of diabetic mothers studied during days 1 to 6 were clinically satisfactory. None of them developed respiratory distress, which is the major danger to these infants in the neonatal period (Gellis and Hsia, 1959), but one of these infants was subjected to the stress of exchange transfusion on both day 1 and day 3 on account of haemolytic disease due to Rhesus incompatibility.

Most of the results presented were obtained by the estimation of Fraction III steroids, both that group of primary hormones retaining the 4-en-3-one group and giving sodium fluorescence, and the quantitatively more prominent group of adrenocortical steroid hormones reducing blue tetrazolium, including both these primary hormones and their metabolites. The estimation of the Fraction II blue tetrazolium-reducing steroid 3β:21-dihydroxypregn-5-ene-20-one in the urine of infants born to diabetic mothers has been described in detail elsewhere (Cathro, Birchall, Mitchell, and Forsyth, 1965).

Results

Group analysis for 17-hydroxycorticosteroids in a few specimens of urine from diabetic and pre-diabetic women in the third trimester of pregnancy showed an excretion no different from that of normal pregnant women. Similarly, analysis of the urine obtained from mothers during the 24-hour period following delivery showed that the excretion levels of 17-hydroxycorticosteroids and of 17-oxosteroids in diabetic and pre-diabetic mothers were within the ranges found in normal women following childbirth. In both diabetic and pre-diabetic patients caesarean section was the mode of delivery for two-thirds of the mothers, and their corticosteroid excretion values during the ensuing 24 hours were not unduly high. The average value for 17-hydroxycorticosteroid excretion obtained from the diabetic women was 14·6 mg./24 hr. and that from the pre-diabetic women, 12·2 mg./24 hr. The respective values for the 17-oxosteroids were 8·2 mg./24 hr. and 9·3 mg./24 hr.

Measurement of the individual neutral steroids excreted by infants showed essentially the same patterns of steroid excretion in infants born to diabetic mothers as in full-term and premature infants born to women without endocrine disease. Although the unique excretion patterns during the first week of life are indicative of a different mode of steroid metabolism from that found in adults (Cathro et al., 1963), the structure of many of the compounds which have been tentatively identified in infancy urine, and the probable structures of others, from theoretical considerations (Birchall, 1963; Cathro, 1964), are such that quantitative estimations of these individual compounds are considered likely to have provided a reasonably accurate indication of adrenocortical activity in early infancy.

Application of this technique revealed that there was much greater individual variation in the quantities of steroids excreted by healthy endocrinologically normal infants than is to be found with adults. In our view, satisfactory comparison of the steroid outputs of different infants may be made on a basis of the excretion per kg. birth weight per 24 hr. Since the steroid excretion patterns of premature infants and of those born at term were found to be similar, it was thought permissible to compare the steroid excretion values per kg. birth weight amongst infants of very different birth weights, irrespective of their estimated maturity at the time of birth. Although subsequent recordings of weight have been available, excretion values have been calculated per kg. birth weight per 24 hr. throughout the first week of life. The steroid excretion results individually determined for each 24-hour sample on days 1, 2,
and 3 have been used to present average 24-hour excretion values during the first three days for each infant. In some instances, results were obtained from the excretion values measured on only two of these days, and occasionally the output measured in an isolated 24-hour urine specimen was charted. This use of an average value for days 1 to 3 was thought justifiable in that the degree of development of renal function might differ in individual infants, and in some cases there might be a delay of at least 24 hours before evidence of increased corticosteroid production was shown in the urinary excretion of steroids.

For each infant, sodium-fluorescent steroids in Fraction III of the urinary extracts were measured individually and totalled. The principal constituents of this group of steroids, in descending order of polarity, are 6β-hydroxy cortisol, a prominent unidentified compound with properties suggesting that it may be 6β-hydroxy corticosterone, cortisol, cortisone, and corticosterone. The total excretion of these compounds in 24 hours during days 1 to 3 is shown in Fig. 1a and b. The one infant born to an established diabetic mother to show a high output was the infant with concomitant haemolytic disease requiring an exchange transfusion on the day of birth and a second exchange at 68 hours. Fig. 1b shows that this high excretion value has been matched on occasion by that of acutely ill premature infants, and approached by the output of one stressed full-term infant whose mother had no endocrine abnormality. The one infant of a pre-diabetic mother to show a high output of sodium-fluorescent steroids was in excellent clinical condition from the moment of birth, and had an output of blue tetrazolium-reducing steroids which was within the normal range. This infant did, however, have a cushingoid appearance and a noticeably reddish colour.

During the first three days of life the sodium-fluorescent steroid excretion values for the other infants born to diabetic and pre-diabetic mothers were found unremarkable, and were lower than those of many of the stressed infants. Although not illustrated, a similar scatter of results for the sodium-fluorescent steroid excretion values from infants of diabetic mothers compared with those from healthy full-term control infants and from premature infants was found on the sixth day of life.

Increased corticosteroid output, or, certainly, a tendency towards increased adrenal activity in the infants born to diabetic mothers is suggested by the
excretion of blue tetrazolium-reducing steroids during the first three days of life, illustrated in Fig. 2a and b. The compounds measured are all the Fraction III steroids reducing blue tetrazolium. Evidence suggests that corticosteroids amongst the compounds measured are: 6β-hydroxylated and other highly polar metabolites of cortisol, tetrahydrocortisol, tetrahydrocortisone, cortisol, cortisone, dihydrocortisone, tetrahydrocorticosterone, allotetrahydrocorticosterone, and corticosterone, as well as other, yet unidentified, steroids. Again, the highest value was obtained from the infant subjected to exchange transfusion. However, none of the other infants of diabetic mothers could be classified as stressed on clinical grounds. Despite a scatter of results amongst those from healthy control infants, Figs. 3 and 4 illustrate that the corticosteroid output of infants born to diabetic women is generally raised, and in the same range as that from full-term and premature infants who have suffered birth asphyxia or other severe stress. In contrast, values obtained from infants born to pre-diabetic women are within the range of normal.

Fig. 3a and b show that, even on the sixth day of life, an undue proportion of the small series of infants born to diabetic mothers have shown an output of blue tetrazolium-reducing steroids in the upper part of the normal range, if not, in fact, above it. The isolated high value of 910 μg/kg. 24 hr. from an infant of a woman with pre-diabetes is not directly comparable with the others, but it is noteworthy in that it represents the day 7 excretion of an infant whose neonatal progress was poor following forceps delivery at 36 weeks, and who had all the manifestations of the respiratory distress syndrome despite a birth weight of 3.1 kg. Pre-eclamptic toxemia had been diagnosed in the mother in a small outlying hospital and her pre-diabetes was only discovered following investigations during the puerperium—prompted by the poor condition of the infant. The highest value of all on day 6 was from an infant of a diabetic mother with no evidence at all of respiratory distress but in whom the characteristic red coloration was marked. One of the other three high values in infants born to diabetic mothers was given by the infant who had an exchange transfusion on the day of birth and again at 68 hours. As previously stated, none of the other infants born to diabetic or pre-diabetic mothers showed any signs of distress which could be detected clinically.

The excretion values for the relatively non-polar Zimmermann-reacting compounds occurring in
Fraction I of the urinary extracts of the infants of different categories during the first three days of life are shown in Table 1. The characteristic compounds obtained in this fraction of urinary extracts from adults, namely, dehydroepiandrosterone, aetiocholanolone, and androsterone are almost inconspicuous in infancy urine, in which the typical Zimmermann chromogens are substances less polar than androsterone (Cathro et al., 1963). An early impression from work on pooled urine that the excretion of Fraction I compounds seemed to be more marked in premature infants (Birchall, Cathro, Forsyth, and Mitchell, 1961) has been sustained.

The excretion of Fraction II Zimmermann chromogens of medium polarity is reported in Table 2. The highest results have been obtained in infants of diabetic mothers.

Discussion

The results of this study indicate that infants born to women with diabetes mellitus show greater adrenocortical activity than control infants. This is

TABLE 1

EXCRETION OF FRACTION I ZIMMERMANN CHROMOGENS DURING DAYS 1-3 (µg./kg. 24 hr.)

<table>
<thead>
<tr>
<th></th>
<th>Full-term Normal</th>
<th>Full-term Stressed</th>
<th>Premature</th>
<th>Mother Diabetic</th>
<th>Mother Pre-diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>26:1 (13)</td>
<td>24:3 (10)</td>
<td>46 (14)</td>
<td>28:9 (8)</td>
<td>60:4 (4)</td>
</tr>
<tr>
<td>Range</td>
<td>11-76</td>
<td>9-1-55</td>
<td>14-95</td>
<td>22-48</td>
<td>22-96</td>
</tr>
</tbody>
</table>

The numbers of infants studied are given in parentheses.

TABLE 2

EXCRETION OF FRACTION II ZIMMERMANN CHROMOGENS DURING DAYS 1-3 (µg./kg. 24 hr.)

<table>
<thead>
<tr>
<th></th>
<th>Full-term Normal</th>
<th>Full-term Stressed</th>
<th>Premature</th>
<th>Mother Diabetic</th>
<th>Mother Pre-diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>30:9 (14)</td>
<td>35:0 (11)</td>
<td>41:9 (12)</td>
<td>54:2 (8)</td>
<td>30:2 (5)</td>
</tr>
<tr>
<td>Range</td>
<td>12-55</td>
<td>6-63</td>
<td>18-61</td>
<td>30-82</td>
<td>18-53</td>
</tr>
</tbody>
</table>

The numbers of infants studied are given in parentheses.
manifest in an increased excretion of reducing corticosteroids of all ranges of polarity and cannot be attributed merely to the prematurity of these infants. The increased output of corticosteroids found during the first three days of life in the admittedly small series of infants studied has not been associated with respiratory distress or with other illness during the neonatal period, since all the infants were in good condition at birth and maintained satisfactory progress thereafter. In Fig. 2a, if the symbol representing the corticosteroid excretion of the child requiring exchange transfusion is excluded, three of the four values from infants of diabetic mothers which are clearly above the normal range were from infants noted to have a cushingoid appearance. The other infant of an established diabetic mother, who showed the classical physiognomy associated with maternal diabetes, had a blue tetrazolium-reducing steroid output of 557 μg./kg. 24 hr., in the upper part of the range obtained from control infants. Some degree of cushingoid appearance was observed in only one of the infants born to carefully controlled pre-diabetic mothers. This infant was the only one in his category who did not have a corticosteroid output during the first days of life which was in the normal range (Fig. 1a) and, even in his case, the high output of sodium-fluorescent steroids was countered by normal excretion of the blue tetrazolium-reducing compounds which are quantitatively more important. One other child born to an untreated pre-diabetic mother had a more obvious cushingoid appearance and developed respiratory distress. The excretion of corticosteroids by this child on day 7 (Fig. 3a) was very high. By far the highest excretion levels measured were in an infant with haemolytic disease born to a diabetic woman and subjected to two exchange transfusions. This is taken to indicate that the high corticosteroid secretion of infants of diabetic mothers, when they are in satisfactory clinical condition, is not maximum and that it increases when there is extraneous stress. This finding in one infant in the present series is therefore in support of the results of Field et al. (1963) who found a corticosteroid excretion in infants born to diabetic women and developing respiratory distress, which was approximately twice that of stressed premature control infants. Additional evidence suggesting increased adrenocortical activity in the infants of diabetic mothers has been provided in a previous study from this laboratory (Cathro et al., 1965), in which it was noted that the urinary excretion of the steroid 21-hydroxyprogrenolonelone, an intermediary compound in a pathway of corticosteroid biosynthesis of peculiar importance in the foetus and the newborn infant, tended to be higher in infants born to diabetic mothers than in most of the controls.

Our finding of an above average output of corticosteroids by the infants of diabetic mothers was not associated with a raised 17-hydroxycorticosteroid excretion in their mothers, nor was there an increase in individual compounds, including 21-hydroxyprogrenolone, in the maternal urines in which they were measured.

This problem of the maternal contribution to the steroids measurable in the cord blood and in the urine of newborn infants has recently been examined by Aarskog (1965) who has not found the mean concentration of total cortisol in the cord plasma of infants born to diabetic mothers to be significantly different from that observed in the cord plasma of normal full-term and premature infants. Aarskog has verified earlier reports that the corticosteroid-binding globulin levels are much lower in cord plasma than in maternal plasma, and has demonstrated that binding of corticosteroids is less developed in the premature infant than in the infant born at term. He has further shown that though the mean ratio of maternal to cord total plasma cortisol is of the order 3 : 1, the mean ratio of maternal to cord unbound cortisol is about 1 : 1. The nine premature infants he studied showed a mean cortisol level of unbound (and, presumably, physiologically active) cortisol which was significantly higher than the mean cord level of unbound cortisol measured in the nine infants born at term. The mean concentration of unbound cortisol in the cord plasma of 11 infants born to diabetic mothers fell between the levels in the two categories of infants born to normal mothers. Aarskog has interpreted his findings to imply that during foetal life an equilibrium exists across the semi-permeable membrane of the placenta, whereby effective cortisol levels in the foetus are in homeostasis with those of the mother. He admits, however, that at delivery, maternal cortisol levels rise so substantially above the protein-binding capacity that there is likely to be considerable transfer to the foetus, and thus his finding of normal cord levels of unbound cortisol in infants born to diabetic mothers does not exclude the possibility of such infants having above-average levels of effective cortisol during intrauterine life.

In Aarskog's study, the possibility of the adrenal gland of the infant contributing significantly to the unbound cortisol levels on either side of the placenta is not discussed and, no doubt through caution, significance has not been attached to the fact that the mean value for unbound cortisol in the plasma of diabetic mothers was found to be slightly less than the mean value obtained from examination of the levels of unbound cortisol in the cord blood of their
infants, though the ratio of unbound cortisol in maternal and cord plasma was approaching unity at 0·93 : 1. The equivalent ratios for mothers and full-term infants and mothers and premature infants were 1·15 : 1 and 1·05 : 1. In only 2 of the 11 cases of diabetic pregnancy examined were the maternal levels of unbound cortisol actually above the cord levels. Aarskog has suggested that the problem of whether the foetus of the diabetic woman produces, or is subjected to, above normal levels of effective cortisol in utero might best be revealed by a study of the unbound plasma cortisol of the mother throughout pregnancy. New significance is therefore given to an earlier study by Plotz, Davis, and Ricketts (1959) who found an increased output of urinary corticosteroids in 11 out of 13 diabetic women during the second trimester of pregnancy, though during the third trimester their average output was comparable with that of normal pregnant women.

Other recent work by Aarskog (1963, 1965) at first sight appears to be in disagreement with the results of the present study. His determination of the cortisol production rates by the isotope dilution method in 8 newborn infants of diabetic mothers and in 5 normal newborn infants, showed that when the results were corrected for body surface area the average cortisol production per square metre per 24 hours in the infants born to diabetic mothers was 26·89 mg. and that of the controls 21·96 mg. He found an overlap of values from both groups of infants and therefore did not think the difference between the mean values significant. It is of note that the tests were carried out on the controls on days 3 and 4 and on the infants of diabetic mothers on days 4 to 6, and even this relatively slight discrepancy in timing may have been enough to affect the results at a period of life when modes of steroid metabolism are undergoing rapid and progressive alteration (Bertrand, Loras, Gilly, and Cautenet, 1963; Cathro et al., 1963). Moreover, the one infant in Aarskog's rather small series showing a low cortisol production rate would, in the present study, have been classed as the child of a pre-diabetic mother. Had our criteria been adopted, of the 7 infants of diabetic mothers he examined, one gave a value in the lower part of the normal range, 3 gave results in the upper part of the normal range, and the remaining 3 gave results well above the maximum control value. Two of the three high values were from infants who showed no clinical evidence of stress, and thus, though Aarskog has been more cautious in his interpretation of results from a small series, his findings are not completely at variance with those now reported. Aarskog carried out his studies later than the third day of life to exclude the steroid contribution from the mother, but there is nothing to suggest that this is any greater from a mother with diabetes than from a normal control. The present study has been carried out on infants newly released from a diabetic environment, and may thus have shown up differences between the two categories of infants, which become less evident day by day over the first week. Moreover, the present study has measured metabolites of corticosterone as well as of cortisol, and there is evidence that corticosterone is of relatively greater importance in early infancy than it is thereafter (Cathro et al., 1963).

The method adopted in this study has revealed quantitative differences, which are quite definite, between the infants of diabetic mothers and control infants with regard to corticosteroid excretion. Qualitative differences have also been detected but these have been more subtle. The excretion patterns of neutral steroids have been found to be basically similar in all the newborn infants studied. Tetrahydrocortisol, a major cortisol metabolite in the adult, is not a prominent constituent of the urine of normal newborn infants during the first three days of life (Bertrand et al., 1963) when a complex of steroids containing a major unknown compound as well as tetrahydrocortisone is characteristically dominant among reducing steroids of this order of polarity (Cathro et al., 1963). However, in the urine of 4 of the infants of diabetic women included in this study, reducing steroid with the mobility of tetrahydrocortisol has been found as prominent as, or more prominent than, the accumulation of steroid in the tetrahydrocortisone area of the chromatograms. The results of the present study cannot be compared directly with those of Grossman, Crigler, and Gold (1963), since these workers hydrolysed their urine specimens with purified β-glucuronidase, and probably measured tetrahydrocortisone uncontaminated either with hydroxylated Δ⁵-pregnene derivatives (Cathro et al., 1965), or with other reducing steroids with a polarity similar to that of tetrahydrocortisone, which are likely to be released from conjugation by sulfatases present in the crude enzyme preparations used in the standard technique employed in this laboratory. Despite these technological differences, it is probably justifiable to state that the results of the present study support the belief of Grossman and his colleagues that either the tetrahydrocortisol to tetrahydrocortisone ratio is greatly increased in the urine of infants whose mothers have diabetes or, at least, that such infants show evidence of a steroid metabolism that is slightly different both qualitatively and quantitatively from that of normal newborn infants.

A further qualitative difference in the steroid
metabolism of infants born to diabetic mothers is suggested by the results in Table 2, in which the total output of Zimmermann chromogens of medium polarity from such infants is shown to be higher than can be attributable to their prematurity. So many of the prominent steroid compounds in this fraction of the urinary extracts from newborn infants are of an unknown nature (Cathro et al., 1963) that there can be no rational speculation as to the specific significance of this finding. One of the dominant Zimmermann-reacting compounds in Fraction II of urinary extracts from newborn infants is the unidentified compound with the R_f value of 0.19 in the Bush chromatography system LB 21/80 at 28°, described in previous papers (Cathro et al., 1963; Cathro et al., 1965), and this may be the same steroid as has been found in urine collected in later childhood (Gupta and Tanner, 1964). However, the output of this particular steroid in the urine of infants of diabetic mothers has been no higher than has been found in the urine of full-term or of premature infants, whether normal or stressed, and the difference in steroid output in infants of diabetic mothers is due to a general increase in other Zimmermann chromogens in this Fraction. This over-all increase in Zimmermann chromogen excretion in the infants of diabetic mothers lends support to the earlier study of Björklund and Jensen (1955) and is a further pointer to some degree of hyperadrenocorticism in these infants.

Osler and Pedersen (1960) have put forward convincing arguments against hyperadrenocorticism being a major cause of the foetal overgrowth in infants of diabetic mothers. They have assigned the changes in body composition in these infants with their increased proportion of fat as being essentially due to an increased supply of glucose being transmitted across the placenta from the mother during gestation (Osler, 1960; Roszkowski and Janczewska, 1964; Milner and Hales, 1965), with resultant hyperinsulinism as the primary endocrine response in the foetus (Pedersen, 1952; Farquhar, 1956b; Baird and Farquhar, 1962).

Although the cushingoid appearance of infants of diabetic mothers may, therefore, be in part due to a relative increase in their total fat, the results of the present study support Farquhar's (1962) belief that the adrenal gland is involved to some extent in producing the complicated endocrine state of these infants. In view of the high perinatal mortality in infants of diabetic mothers, it is tempting to speculate that their increased adrenal activity reflects an adaptation to stress in the form of some subtle biochemical abnormality, for example, as a response to the foetal hyperinsulinism. In addition, all 4 infants of diabetic mothers on whom electrolyte studies were performed as an ancillary part of the present study showed an above-average excretion of sodium, chloride, and potassium during the first three days and on the sixth day of life, suggesting an alteration in body composition.

Whatever the cause of the hyperadrenocorticism in these infants, evidence of its existence indicates that corticosteroid therapy has no useful place in their management. It is suggested that on the relatively rare occasions when such infants develop symptoms in association with hypoglycaemia, glucagon would be the rational adjuvant to therapy with glucose.

All the infants of diabetic mothers included in this study were in good condition at birth and progressed well thereafter. Only 4 of the 8 demonstrated some degree of the cushingoid appearance which typified such infants prior to the very careful management of diabetes during pregnancy, which is now standard practice (Gellis and Hsia, 1959; Brandstrup, Osler, and Pedersen, 1961; Deul and Durand, 1961), and 3 of these had a high excretion of corticosteroids. One of the infants born to a pre-diabetic woman, whose underlying disorder had not been diagnosed during her pregnancy, showed a very high output of corticosteroids during recovery from respiratory distress. On the other hand, the corticosteroid excretion of infants of pre-diabetic women who were strictly controlled during pregnancy was consistently within the normal range, which provides further circumstantial evidence that strict control of diabetes and pre-diabetes during pregnancy is a major factor in reducing the endocrine abnormality in the foetus.

**Summary**

The urinary excretion of individually estimated neutral steroids on days 1, 2, 3, and 6, from 8 infants born to diabetic mothers, and from 6 infants born to pre-diabetic mothers, has been compared with the excretion of these compounds by full-term and by premature infants born to non-diabetic mothers.

The results suggest that infants born to women with diabetes show adrenocortical activity that is greater than that of control infants. This is manifest in an increased excretion of blue tetrazolium-reducing corticosteroids of all ranges of polarity. This increased excretion of corticosteroids shown by the infants of diabetic mothers is not attributable to their relative prematurity nor to clinically detectable stress.

While differences in the excretion of corticosteroids between infants of diabetic mothers and control infants have been essentially quantitative, more subtle qualitative differences have also been detected,
but these defy interpretation in the present state of knowledge.

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Appendix

The following terms have been used throughout the text.

Aetiocholanolone 3α-hydroxy-5β-androstan-17-one
Allotetrahydrocorticosterone 3α : 11β : 21-trihydroxy-5α-pregn-20-one
Androsterone 3α-hydroxy-5α-androstan-17-one
Blue tetrazolium 3 : 3‘-dianisole-bis-4’ : 4‘ (3 : 5 diphenyl)-tetrazolium chloride
Corticosterone 11β : 21-dihydroxyprog-4-ene-3 : 20-dione
Cortisol 11β : 17α : 21-trihydroxyprog-4-ene-3 : 20-dione
Cortisone 17α : 21-dihydroxyprog-4-ene-3 : 11 : 20-trione
Dehydroepiandrosterone 3β-hydroxy-androst-5-ene-17-one
Dihydrocortisone 17α : 21-dihydroxy-5β-pregn-3 : 11 : 20-trione
6β-Hydroxy cortisol 6β : 11β : 17α : 21-tetrahydroxyprog-4-ene-3 : 20-dione
6β-Hydroxy corticosterone 6β : 11β : 21-trihydroxyprog-4-ene-3 : 20-dione
21-Hydroxypregnolone 3β : 21-dihydroxyprog-5-ene-20-one
Tetrahydrocorticosterone 3α : 11β : 21-trihydroxy-5β-pregn-20-one
Tetrahydro cortisol 3α : 11β : 17α : 21-tetrahydroxy-5β-pregn-20-one
Tetrahydrocortisone 3α : 17α : 21-trihydroxy-5β-pregn-11 : 20-dione


