Adrenocortical Response to Stress in Newborn Infants

D. METHVEN CATHRO,* CONSTANCE C. FORSYTH,† and JENNY CAMERON
From the Department of Child Health, University of Dundee

More than half of the deaths occurring under the age of 12 years take place during the first week of life, and the term 'biochemical death' is sometimes used where an adequate pathological explanation cannot be found at necropsy. Following the pioneer work of Selye (1936), which drew attention to the prominent role of the adrenal cortex in the endocrine response to stress, it became established that an increased secretion of corticosteroids occurred in adults under conditions of stress, and that, in some unknown manner, such hormonal activity was essential to the well-being and even to the survival of the subjects in such circumstances.

In paediatric circles, however, it was thought that the adrenocortical response to stress might be relatively deficient in newborn infants, and Bongiovanni (1951) cited several publications which suggested that the excretion of corticosteroids, measured by methods then available, was lower during the first few days of life than at any other age, if the results were compared on the basis of excretion per unit of surface area.

Measurement of corticosteroid excretion in newborn infants by group methods of assay in instances of acute stress has given inconsistent results. Ulstrom and his colleagues (Ulstrom et al., 1959; Colle et al., 1960) found that the urinary excretion of Porter-Silber chromogens by infants undergoing surgery during the first 4 days of life showed no significant increase over the excretion of these compounds in normal controls, though there was a marked increase in the excretion of 17-hydroxycorticosteroids measured in this way when infants were subjected to surgery during the second week of life. Studies of plasma corticosteroids measured as Porter-Silber chromogens by Klein, Fortunato, and Papadatos (1954) showed that the normal newborn infant from the second to the fifth day of life had a lower level of Porter-Silber chromogens than normal subjects aged 3 weeks to 32 years.

In contrast, Venning, Randall, and Gyorgy (1949) found that premature infants with atelectasis and respiratory embarrassment born to diabetic mothers excreted greater quantities of glucocorticoids than did healthy premature infants. The difference was attributed to the respiratory distress, though the maternal diabetes may have been a factor (Cathro and Forsyth, 1965). Hillman (1961) estimated the Porter-Silber chromogens in the urine of infants born to women with no endocrine disease and found that the urinary excretion of these compounds during the first 3 days of life was, on average, twice as high in infants with the respiratory distress syndrome as it was in normal controls. However, when the position was reviewed in 1963, Bongiovanni considered that it had not yet been ascertained whether a brief period of relative adrenocortical insufficiency occurred about the second or third day of life.

Some of the doubt regarding the adequacy of suprarenal function had arisen from knowledge of the anatomy of the adrenal glands at birth and immediately thereafter, since the large fetal cortex degenerates rapidly after birth, though the small adult cortex gradually increases in thickness. However, recent work has pointed to the fetal zone being concerned essentially with the elaboration of oestrogen precursor Δ⁴-steroids from pregnenolone supplied by the placenta, and it is believed that atrophy of the fetal zone results from the placental connexion being severed. After birth, only the adult zone of the adrenal cortex can synthesize biologically active Δ⁴-corticosteroids, as it possesses the essential Δ⁴-3β-ol-dehydrogenase activity, whereas the fetal cortex does not. The adult cortex therefore must supply all the needs of the infant for the period of adaptation to extrauterine life. However, the high excretion of Δ⁴-steroids in the urine during the first week of life (Cathro et al., 1963, 1965; Mitchell, 1967) is evidence that there is a relative insufficiency of Δ⁴-3β-ol-dehydrogenase in the definitive cortex at this period, which does not exist
in later life. On theoretical grounds, therefore, because of enzyme immaturity, the adrenal glands in the newborn infant might fail to provide as adequate a reserve of function under conditions of stress as is shown by adults.

**Materials and Methods**

In 1958, we undertook a study of the urinary excretion of adrenal steroids in 31 male full-term infants and 13 male premature infants, to determine the adrenal response to stress in the newborn period. In 1966, the work was extended to include 18 male dysmature or 'light-for-dates' infants. Complete 24-hour urine samples were collected on the first, second, third, and sixth days of life. Infants were classified as full-term and premature according to well-recognized definitions, but no dysmature infants were included in either of these two groups. The premature group, therefore, was truly immature in that the infants were all of expected weight for the period of gestation. For the dysmature group we used the criteria of Crosse (1957) and Gruenwald (1964).

In Fig. 1 the distribution of the birthweights of the dysmature infants is compared with the data of these authors. Using the Gruenwald criteria, 7 were between 1 and 2 SD below the expected weight for gestational age, and 11 were more than 2 SD below.

Infants were classified as normal or stressed according to the clinical course. The normal ones behaved as healthy infants. The stressed full-term infants included those with asphyxia before or during birth, and also those suffering from Rhesus incompatibility requiring exchange transfusion. The premature and dysmature infants classified as stressed suffered from asphyxia before and during birth or from the respiratory distress syndrome. Death occurred in 1 of the 12 stressed full-term infants, in 1 of the 5 stressed premature infants, and in 3 of the 9 stressed dysmature infants.

Originally, block assay techniques were applied to the urine, but they were found to be unsatisfactory, and paper chromatography as described by Bush and Willoughby (1957) and Bush and Mahesh (1959) was adopted to measure the individual steroids. Considerable modification of Bush’s method for adult urine was required before good results were obtained with infant urine. In the method finally adopted (Birchall et al., 1961, 1963), the neutral steroids are extracted from urine after enzyme hydrolysis designed to release both glucuronides and sulphates. It was soon found that the pattern of steroid excretion in newborn infants was quite different from that in adults, and this was reported by Cathro et al. (1963, 1965). The present report is concerned only with the corticosteroid fraction as separated by Bush preliminary chromatograms. The individual steroids reducing blue tetrazolium and those giving sodium fluorescence were measured, and the results, expressed as total blue tetrazolium-reducing steroids and as total sodium-fluorescent steroids, were obtained by adding together the quantities of the various individual steroids giving these reactions. Of the blue tetrazolium-reducing steroids, tetrahydrocortisol and tetrahydrocortisone were found in small amounts. Other reducing steroids included very polar compounds believed to be 6β-hydroxylated corticosteroids, while compounds apparently preserving the Aβ structure of biosynthetic intermediates were also measured in considerable quantities. The steroids giving sodium fluorescence were thought to include 6β-hydroxycortisol, 6β-hydroxycorticosterone, cortisol, cortisone, and corticosterone.

**Results**

From current information on suprarenal metabolism in the newborn period, we think it a reasonable supposition that the blue tetrazolium-reducing and sodium-fluorescent steroids, known and unknown, measured by our method, are largely derived from the infant adrenal glands and are likely to reflect their secretory activity. We have found that the possible contribution from the maternal adrenal glands or the placenta has not been sufficient to lead to an over-all reduction in the excretion of these steroids by healthy infants on the sixth day of life, compared with the excretion on the first three days, suggesting that they are chiefly of infant and not maternal origin. Furthermore, unpublished studies have shown no correlation between the excretion of corticosteroids by infants and by their mothers in the 24 hours after delivery (Cathro, 1964).
TABLE

Steroid Assay | Day(s) | Full-term Normal | Full-term Stressed | Premature
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Total blue tetrazolium reducing steroids</td>
<td>Average of days 1, 2, 3</td>
<td>386</td>
<td>121-651</td>
<td>641</td>
</tr>
<tr>
<td>Cortisol</td>
<td>Day 6</td>
<td>322</td>
<td>118-665</td>
<td>523</td>
</tr>
<tr>
<td>Cortisone</td>
<td>Average of days 1, 2, 3</td>
<td>51</td>
<td>14-154</td>
<td>79</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>Average of days 1, 2, 3</td>
<td>7-9</td>
<td>1-2-20-7</td>
<td>6-9</td>
</tr>
</tbody>
</table>

Fig. 3 resembles Fig. 2, but here the results are plotted against gestational age. Infants born before term show a steroid excretion falling within the range shown by those born at or near term.

In order to compare the results in infants of low and normal birthweight, we decided to express the excretion in μg./kg. birthweight per 24 hours. The mean excretion and the range of excretion are given numerically in the Table in the Appendix.

Fig. 4 illustrates the tendency towards a higher excretion of blue tetrazolium-reducing steroids in response to stress during the first 3 days of life in full-term and in premature infants. The response is less definite in the dysmature group. Certain stressed infants of all categories show low or normal values.

Fig. 5 shows that on the sixth day of life the tendency to a higher excretion in stressed infants is still apparent, but by that day the number of...
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(stressed infants was small, as some had died while others had fully recovered clinically and were therefore classified as normal. The distribution of the results from stressed dysmature infants is of particular interest in that one of these infants gave the lowest value recorded from any stressed infant on this day, though 3 showed a high excretion.

Fig. 6 shows that there is a tendency for an increased excretion of sodium-fluorescent steroids in stressed infants during the first 3 days of life, but the rise is less obvious than with the blue tetrazolium-reducing steroids.

On the sixth day also the average value for the excretion of sodium-fluorescent steroids by stressed infants is higher, though, in parallel with the results for the first 3 days, the difference is less definite than with the blue tetrazolium-reducing steroids (Fig. 7).

The results for urinary cortisol alone are given in Fig. 8, and a rise in some stressed infants of the premature and dysmature group is seen, though no rise is apparent in the stressed full-term infants. This may be because the enzyme system for the conversion of cortisol to tetrahydro-derivatives is more active in full-term than in premature infants.

<table>
<thead>
<tr>
<th>Normal</th>
<th>Premature Stressed</th>
<th>Dysmature Normal</th>
<th>Dysmature Stressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>204-544</td>
<td>818</td>
<td>343-1195</td>
<td>429</td>
</tr>
<tr>
<td>207-766</td>
<td>789</td>
<td>626-952</td>
<td>366</td>
</tr>
<tr>
<td>16-93</td>
<td>99</td>
<td>34-195</td>
<td>55</td>
</tr>
<tr>
<td>16-86</td>
<td>82</td>
<td>74-89</td>
<td>45</td>
</tr>
<tr>
<td>1-7-11-2</td>
<td>12-9</td>
<td>2-7-22-5</td>
<td>4-7</td>
</tr>
<tr>
<td>1-9-29-0</td>
<td>17-9</td>
<td>3-8-66-6</td>
<td>7-8</td>
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<tr>
<td>1-6-18-2</td>
<td>14-9</td>
<td>9-5-22-8</td>
<td>17-6</td>
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</tbody>
</table>

![Graph](image-url)
Fig. 6.—*Average excretion of total sodium-fluorescent steroids on days 1, 2, and 3.*

Fig. 9 shows that certain infants show a rise in cortisone excretion in response to stress.

Fig. 10 illustrates that several full-term infants show a rise in corticosterone excretion in response to stress, and 2 premature and 2 dysmature infants also show high readings. It might be emphasized at this point that in full-term normal infants the excretion of corticosterone as estimated by our method, which measures urinary corticosterone sulphate as well as the free steroid, was double that of cortisol or cortisone, suggesting a greater relative importance of corticosterone or of corticosterone...
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sulphate in the steroid metabolism of the newborn infant than in the older child or adult.

Discussion

It is well recognized in the older child or the adult that the urinary excretion of unmetabolized 'cortisol' is a much better guide to adrenocortical production of cortisol than is the urinary excretion of cortisol metabolites as measured by block assays. The present study shows that measurement of cortisol excretion by our method is not a satisfactory guide to adrenocortical activity in the newborn infant, and the same applies to measurement of the other primary steroids that are excreted in urine, namely cortisone and corticosterone. However, the quantities of these three steroids excreted by newborn infants were small, and it is not surprising that measurement of the total sodium-fluorescent steroid excretion has given a clearer picture of the adrenocortical response to stress.

The blue tetrazolium-reducing steroids gave the best indication of stress provoked an increased excretion of steroids in several infants in each of the three categories studied. These steroids are present in the urine of infants in approximately 8 times the quantity of the sodium-fluorescent steroids and the results are therefore less liable to error. In addition, by the nature of our method, the measurement of blue tetrazolium-reducing steroids is more accurate. Nevertheless, it has been our experience that the excretion levels of the two groups of compounds in any one infant tend to be in parallel.

When the values for the excretion of blue tetrazolium-reducing steroids/kg. per 24 hours, averaged for the first 3 days of life, in full-term normal infants and clinically satisfactory premature and dysmature infants, were compared statistically, no difference between the groups was apparent. Likewise, results based on the measurement of sodium-fluorescent steroids did not show any statistically significant difference between the groups. The resting level of excretion in our series was thus of the same order in full-term, premature, and dysmature infants. Our work, therefore, does not support the concept of frank hypo-adrenocorticism in premature or dysmature infants compared to full-term infants.

When the results suggesting an increased output of blue tetrazolium-reducing steroids in the first 3 days of life in response to stress were submitted to statistical analysis by the t-test, the full-term group as a whole showed a response which was statistically significant, the probability of error being 0.001 or 1/1000. The response in the premature group was also statistically significant though less definitely so, the probability of error being 0.01 or 1/100. The response in the dysmature group was not statistically significant. From Fig. 4 it will be noted that one normal dysmature infant showed a very high output and as he became ill on the 4th day of life the figures were also submitted to analysis after classifying him as stressed, but the results again showed no significant difference for the dysmature group. Thus, the dysmature group of infants considered as a whole showed no significant response to stress during the first 3 days of life.

When the blue tetrazolium-reducing steroid output on the sixth day (Fig. 5) was submitted to statistical analysis, the stressed full-term group of infants showed a rise in excretion which was just statistically significant, the probability of error being 0.05 or 1/20. The number of premature infants studied on the sixth day was too small for statistical analysis; the dysmature group of infants showed no significant difference.

In summary, our results have shown that certain infants of the full-term, premature, and dysmature categories have shown an increased excretion of blue tetrazolium-reducing and sodium-fluorescent steroids in response to stress, but that other infants in all 3 categories have not. When the excretion of blue tetrazolium-reducing steroids has been considered in each group as a whole, the full-term group has shown a statistically significant response to stress on days 1, 2, and 3 and on day 6, and the
premature group has shown a statistically significant response on days 1, 2, and 3, the numbers on day 6 being too small for statistical analysis. The dysmature group has shown no statistically significant response to stress either on days 1, 2, and 3 or on day 6.

Measurement of cortisol production rates in newborn infants by other workers has supported the concept of adequate adrenocortical function in infants of normal birthweight born at term. Aarskog (1965) found a mean cortisol production rate of 22 mg./sq. m. per 24 hours in 5 full-term infants studied on the third day of life. For comparison, an approximate figure for the cortisol production rate in adult males is of the order of 13–14 mg./sq. m. per 24 hours (Dorfman and Ungar, 1965). Bertrand et al. (1963a, b) have also found the cortisol production rate of newborn infants to be above that of adults, their infant values averaging 22·2 mg./sq. m. per 24 hours in the first 5 days of life. Their level for 5 healthy subjects between the ages of 5 and 52 years was 12·4 mg./sq. m. per 24 hours. Similar results from a number of studies by Kenny, Malvaux, and Migeon (1963), Kenny, Preeyasombat, and Migeon (1966b), and Kenny et al. (1966a, 1966c) have also shown that newborn infants less than 5 days of age secrete more cortisol per sq. m. body surface than do older infants, children, or adults. It may be postulated that the raised cortisol production rate of healthy normal infants during the first few days of life signifies an adrenocortical response to normal adaptation to extrauterine life.

The low birthweight infant, prematurely born, but of expected weight for gestational age, also appears to have a satisfactory cortisol production rate (Kenny et al., 1963, 1966a, c), and recent work based on the urinary excretion of corticosteroids has suggested that the hypothalamo-hypophysial-adrenal axis of the premature infant can respond to metyrapone administration (Klein et al., 1962).

No cortisol production rate studies have been carried out on full-term and premature newborn infants undergoing physical stress more severe than that imposed by an uneventful delivery and neonatal course. Our assessment of the capacity of the adrenal cortex to respond to pathological stresses in the first week of life provides evidence that the full-term and premature groups of infants show a response that is statistically significant, whereas the dysmature group does not.

The results in the group of dysmature infants are not unexpected on theoretical grounds. It is well established that the function of the fetal zone of the adrenal cortex in utero may be depressed in pre-eclamptic toxemia, and that the resultant low oestriol excretion in the mother's urine provides an early indication of fetal deprivation. It is believed that the enzymes in the fetal zone of the adrenal cortex are particularly sensitive to oxygen lack, and this deficit may be rectified on delivery. These infants are also generally undernourished in utero. This is a further possible cause for underactivity of the fetal zone and, in all probability, for an associated hypofunction in the definitive or adult cortex, the zone required for the adaptation response after birth. It is tempting to postulate that those dysmature infants showing a response to postnatal stress have been less deprived than others in whom prolonged adrenocortical hypofunction in utero may have given rise to a situation in which the adrenocortical response to postnatal asphyxia or other stress may be suboptimal.

Recently, Kenny and Preeyasombat (1967) have published cortisol production rate studies on 8 dysmature infants, all of whom had symptomatic hypoglycaemia during the first 4 days of life. These workers found the cortisol production rate per kg. body weight to be more than 2 SD below the normal mean in 3 of the infants, between 1 and 2 SD below in a fourth, and significantly above the mean in only 1 infant. In our series of dysmature infants only 2 had symptomatic hypoglycaemia: 1 of these infants had the lowest steroid excretion of any stressed baby studied, while the other showed an above average steroid excretion.

In conclusion, our results indicate that there is no evidence of frank hypoadrenocorticism in premature and dysmature infants pursuing a normal clinical course compared to full-term infants. The majority of full-term and premature infants show an above average excretion of corticosteroids in response to stress. However, certain infants in all three categories may fail to show a response to a stressful situation, while the dysmature group of infants shows an inadequate response more frequently than the other two groups to the extent that, taking the group as a whole, there is no statistically significant response to stress.

In therapy, therefore, while physiological doses of cortisol of the order of 5–7½ mg. daily might occasionally be of value in situations of stress in full-term and premature infants, such therapy might be indicated more often in the management of dysmature infants whose response to stress is more likely to be suboptimal.

Summary

The excretion of individual corticosteroids in the urine of male newborn infants averaged for the first, second, and third days of life and for the sixth day
of life has been studied by the method described by Birchall et al. (1963). 31 full-term, 13 premature, and 18 dysmature infants have been included, and the results compared in those pursuing a normal clinical course and those undergoing various types of stress. The steroids have been measured individually and then summed to give the total blue tetrazolium-reducing steroids and the total sodium-fluorescent steroids.

Results showed a wide range of steroid excretion by normal infants. Associated with stress, an increased steroid output was apparent in some infants of all 3 groups, indicating a response of the adrenal glands in the first few days of life. When the groups were considered as a whole, this response, measured in terms of the blue tetrazolium-reducing steroids, was statistically significant in the full-term and premature infants, but not in the dysmature infants.

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REFERENCES


Appendix

The following terms have been used in the text.

<table>
<thead>
<tr>
<th>Trivial Name</th>
<th>Compound</th>
</tr>
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<tbody>
<tr>
<td>Cortisol</td>
<td>11β:17α:21-trihydroxy-pregna-4-ene-3:20-dione</td>
</tr>
<tr>
<td>Cortisone</td>
<td>17α:21-dihydroxypregna-4-ene-3:11:20-trione</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>11β:21-dihydroxypregna-4-ene-3:20-dione</td>
</tr>
<tr>
<td>Tetrahydrocortisol</td>
<td>3α:11β:17α:21-tetrahydroxy-5β-pregn-20-one</td>
</tr>
<tr>
<td>Tetrahydrocortisone</td>
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</tr>
<tr>
<td>6β-hydroxycortisol</td>
<td>6β:11β:17α:21-tetrahydroxy-pregna-4-ene-3:20-dione</td>
</tr>
<tr>
<td>6β-hydroxy-corticosterone</td>
<td>6β:11β:21-trihydroxy-pregna-4-ene-3:20-dione</td>
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