

# Urinary excretion of cortisol after immunisation

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## Abstract

Urinary cortisol excretion and rectal temperature were measured in 66 infants before and after immunisation against diphtheria, tetanus, pertussis, and *Haemophilus influenzae* type b. Immunisation produced a significant increase of rectal temperature the next night at all ages. Infants without an adult-like night time body temperature pattern had a significant increase in urinary cortisol excretion night and morning after immunisation. Once an adult-like night time body temperature pattern developed immunisation no longer significantly raised urinary cortisol output.

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Immunisation leads to a number of physiological responses, some of which may be related to the effectiveness of the immunisation process. We have already shown that immunisation of infants against diphtheria, tetanus and pertussis (DTP) produces a significant disturbance of sleeping body temperature the next night.<sup>1</sup> Body temperature fails to fall in the normal way, though febrile levels are rarely attained. A very similar pattern of temperature disturbance occurs during the early, incubation phase of minor viral illnesses,<sup>2</sup> and in individual babies there is a strong correlation between the temperature disturbance after immunisation and that which occurs when the infant is ill. This suggests that the temperature change is more a property of the host response than the infecting agent.

In this study we investigated another feature of the host response – the secretion of glucocorticoids from the adrenal cortex – by measuring the urinary excretion of cortisol in infants the night and morning after routine immunisation against DTP and *Haemophilus influenzae* type b. We have already studied the development of urinary excretion of cortisol and its relationship to the development of body temperature patterns, and shown that the onset of an adult-like temperature pattern is associated with an abrupt increase in the difference between night time and morning excretion of cortisol.<sup>3</sup>

## Methods

Subjects were recruited soon after birth from infants born in the Leicestershire maternity hospitals. Informed parental consent was obtained to monitor infants at home once a week for each week from 6 to about 16 weeks of age, and for the night after at least one of the first two immunisation against DTP and

*H influenzae* type b scheduled at about 8 and 12 weeks of age. Full perinatal data were collected.

At each visit a health visitor trained in the techniques weighed the baby naked and inserted a soft probe 5 cm from the anal margin to monitor rectal temperature via a Grant Squirrel data logger, set to sample at one minute intervals throughout the night. A second probe connected to the same logger monitored room temperature. At bedtime a urine bag (Hollister U-bag), modified to reduce the risk of detachment, was attached to the infant, and urine collected overnight until the first feed the next morning. At that time the bag was drained into a sample container, and then left attached to collect a second sample of urine for a further four hours. Urine samples were deep frozen within four hours and subsequently analysed for creatinine by standard techniques and for cortisol by extractive radioimmunoassay (Coat a Count assay). Parents were provided with a diary to record, prospectively, all events involving the baby such as feeds, nappy changes, crying, etc.

Data from the loggers were downloaded to computer for analysis. Data were inspected for evidence of problems such as lost probes, and only unblemished data analysed further.

Ethical permission was obtained for these studies. Statistical comparisons were made by Student's *t* test. Correlation was examined by product moment correlation.

## Results

### SUBJECTS

Sixty six infants were successfully studied before and after at least one immunisation. Of

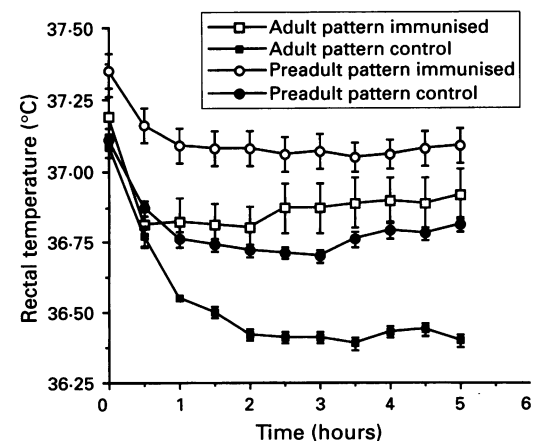


Figure 1 The overnight pattern of rectal temperature before and after immunisation against DTP and *H influenzae* type b in babies who have and have not developed an adult-like night time pattern of rectal temperature. Points show mean (SE); time zero is bedtime. All points represent data from at least 20 babies.

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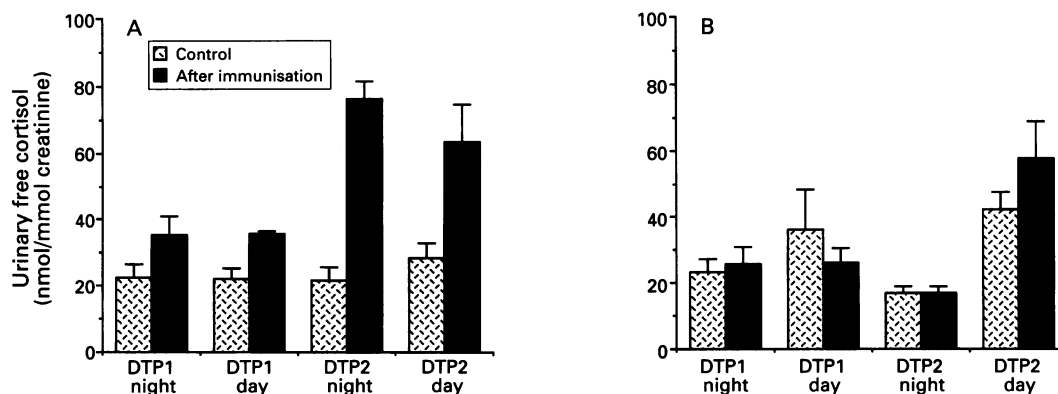


Figure 2 Urinary excretion of cortisol, expressed per mmol creatinine to allow for variations in urine flow. (A) The night and morning before and after immunisation against DTP and H influenzae type b for the first and second time in babies who have yet to develop an adult-like night time body temperature pattern;  $n=31$  for DTP1 data,  $n=6$  for DTP2 data. After DTP1 cortisol excretion is significantly increased ( $p<0.01$ ,  $t$  test) both night and morning. After DTP2 cortisol excretion is significantly ( $p<0.02$ ,  $t$  test) increased both night and morning. The levels of cortisol excretion night and morning are significantly higher after DTP2 than DTP1 ( $p<0.05$ ,  $t$  test). (B) The night and morning after immunisation against DTP and H influenzae type b for the first and second time in infants who have developed an adult-like night time body temperature pattern;  $n=8$  for DTP1,  $n=22$  for DTP2. There are no significant differences between control and postimmunisation samples.

these, 39 were studied after the first DTP and 28 after the second. Forty six (70%) were boys. The mean (SEM) birth weight was 3534 (76) g, with a gestation of 39.4 (0.4) weeks; maternal age was 28.4 (0.6) years. Thirteen of the infants were first babies, 25 had two or more siblings. The social class distribution of the infants resembled that of the Leicestershire population as a whole.

#### BODY TEMPERATURE PATTERNS

These infants developed night time patterns of body temperature as we have described previously.<sup>4,5</sup> In any infant sleeping body temperature fell to about 36.8°C with sleep for the first few weeks of recording, then suddenly between one week and the next temperature began to fall more with sleep at night to about 36.3°C, an adult-like pattern. The transition between these two temperature patterns occurred at different ages in different babies, but on average occurred at 11 (0.5) weeks. Once this pattern was established it was maintained unless the baby was ill or immunised.

Figure 1 shows that, as in our previous studies, body temperature patterns are significantly disturbed after immunisation, both in babies who have yet to establish an adult-like body temperature and in those that have done so. This disturbance of body temperature occurs despite the fact that 72% of the infants were given paracetamol by their parents at or near bedtime.

Figure 2 compares the urinary excretion of cortisol, expressed per mmol creatinine to allow for variation in urine flow, before immunisation, the night after immunisation, and the morning after immunisation. Data were analysed separately for babies who had yet to develop their adult-like body temperature pattern and those who had developed it, because we have already established that the pattern of urinary excretion of cortisol changes as the adult-like body temperature pattern occurs.<sup>3</sup>

First immunisation significantly increased cortisol excretion both night and morning in

infants who had yet to develop an adult-like temperature pattern ( $p<0.01$ ,  $t$  test in each case). The mean (SEM) urinary free cortisol concentrations for babies who had yet to develop an adult-like body temperature pattern were: first DTP, night time control 22.3 (4.1) and after immunisation 35.5 (5.3); first DTP, daytime control 22.1 (3.0) and daytime after immunisation 35.9 (0.7) nmol/mmol creatinine. Second DTP, night time control 21.6 (4.0) and after immunisation 76.3 (5.2); second DTP, daytime control 28.4 (4.5) and after immunisation 63.5 (11.0) nmol/mmol creatinine. The effect of second immunisation was therefore even greater (significant at  $p<0.01$ ,  $t$  test both night and morning). Once the adult-like pattern of temperature had developed, however, immunisation had no significant effect on cortisol excretion either night or morning. As with the body temperature disturbances we have reported before, there was considerable individual variation in the cortisol excretion. To establish whether this was related to the body temperature disturbance we correlated the morning and night time urinary cortisol excretion separately with the maximum body temperature of the infant 2–5 hours after bedtime the night of immunisation; a time when it would normally be at its lowest. There were, however, no significant correlations between cortisol excretion and temperature disturbance.

#### Discussion

We have shown that excess cortisol appears in the urine of young, but not older, infants during the 24 hours after immunisation. Previous studies have examined the salivary cortisol concentrations very soon (20 min) after immunisation, but we can find no reports of longer term measurements in urine. The stress of injection itself is likely to lead to increased adrenocortical secretion, as reported previously, but it seems unlikely that this stress will be responsible for continuing excretion over many hours. The secretion of cortisol is,

however, an established part of the host response to an immunological challenge. Its secretion is stimulated by various mediators which act, among other things, to increase body temperature. We have observed this change in body temperature the night after immunisation. As the injections were almost always given in the morning in this study, there was a delay of around 12 hours in the temperature response, which occurred despite routine treatment with paracetamol. It may be, therefore, that the later excretion of cortisol we have measured reflects another part of this same response. There is no correlation between the extent of temperature disturbance and the extent of increase in cortisol excretion, which might have been expected. For babies who have yet to develop an adult-like temperature pattern there is considerably greater excretion of cortisol after the second immunisation. There is no reason to suppose that the stress of injection is any greater second time around, so this may reflect a greater response to the immunisation.

There are significant developmental changes in the cortisol response. It has been reported previously that older infants show a smaller rise in salivary cortisol immediately after injection. In this study, the older infants, who had developed an adult-like body temperature pattern, did not increase cortisol excretion after either first or second immunisation. It seems unlikely that injections as such suddenly become less stressful, so this may reflect a change in the host response to immunisation. The change from responding to not responding is much more strongly correlated with the development of an adult-like body temperature pattern than it is with age. If babies are grouped by age band (for example, more and less than 12 weeks

old), then the same general pattern is apparent, except that some older babies respond to immunisation by increasing cortisol output. When examined closely, virtually all of these cases are where the baby has yet to develop an adult-like temperature pattern.

This change in the response with the development of adult-like body temperature patterns could be of considerable significance. It implies first, that the host response to an immunological challenge alters at the time that an apparently unrelated physiological system matures. Second, as adult-like body temperature patterns develop at considerably different ages in different babies, it means that some infants will remain in the infantile stage of immunological development, as far as cortisol excretion is concerned, for much longer than others.

The developing infant therefore undergoes a series of changes that may radically affect the way in which it will cope with infection, and individual differences in this developmental process may underlie differences in vulnerability between different infants.

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