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pH changes in the dermis during the course of the tuberculin skin test

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SUMMARY

The response of six healthy young adults to tuberculin skin testing was studied. Five subjects developed a typical delayed-type hypersensitivity reaction to PPD with a local rise in skin temperature, and the sixth showed a less intense response; a considerable increase in blood flow velocity was seen in all reactions. All subjects showed a fall in pH in the dermis during the course of the reaction: in four subjects the pH minimum occurred at the time when the changes of erythema and induration were most prominent, in one subject the pH fall preceded the maximal clinical changes, and in the remaining subject a substantial fall in pH occurred with only transient erythema. It was concluded that the local tissue acidosis had resulted from the greatly increased metabolic demand of the lymphocytes and monocytes attracted into the dermis as part of the type IV delayed-type hypersensitivity reaction, and that the concurrent reactive hyperaemia was insufficient to clear the acidic metabolic products of the greatly increased cell population.

INTRODUCTION

Skin tests are the classical method for the detection of type IV delayed-type hypersensitivity (DHS) reactions. They are widely used to determine the state of immunity of patients in clinical practice and population surveys. However, these tests have also proved to be a valuable experimental system for investigation of the pathogenesis of such reactions and for elucidation of the pathophysiology of chronic inflammation in which immunological type IV reactions often have a dominant role.

The details of the micro-anatomical relations between cells of different types at the site of a type IV reaction are currently being elucidated with immunocytochemical methods employing monoclonal antibodies to various phenotypic subsets (Poulter *et al.*, 1982; Scheynius, Klareskog & Forsum, 1982; Platt *et al.*, 1983). It is now clear that the numbers of cells infiltrating into the dermis at the site of the tuberculin reaction in man is very large (Gibbs *et al.*, 1984; Beck *et al.*, 1986a): we estimate that the density of cells in the dermis at the centre of a fairly intense lesion in man increases about 100-fold (unpublished observations).

This focally dense cellular infiltrate is accompanied by a substantial increase in blood flow velocity in the dermal microcirculation (Beck & Spence, 1986), but it is not clear whether the perfusion rate is sufficiently great to compensate fully for the extra metabolic products produced by the increased numbers of cells in the tissue. If the hyperaemia were inadequate, then it is likely that a local acidosis would arise. Recently, a robust glass semimicro-electrode was developed for tissue pH measurements in a clinical setting (Harrison & Walker, 1977, 1979, 1980). This electrode was used in the present study to evaluate the relationship between local blood flow and tissue pH changes throughout the time–course of the tuberculin DHS reaction in normal subjects.

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MATERIALS AND METHODS

Subjects

Six healthy young adult doctors and students (five men and one women, aged 22–32 years) participated in the study; all gave informed consent without coercion. The investigation had been approved by the University of Dundee Ethics Committee. Skin tests were performed on the volar aspect of the forearm by intradermal injection of 0·1 ml tuberculin purified protein derivative BP (PPD, Evans Medical Ltd, Beaconsfield, Bucks) solution 1 : 1000.

Assessment of skin test response

The site was examined daily and, following a 10 min equilibration period with the forearm exposed to air in a room at $21 \pm 0{\cdot}5°$, the diameters of the areas of erythema and induration were measured with a ruler in the longitudinal and transverse axes of the forearm. In clinical terms, the reaction was

considered to be 'positive' if induration was > 5 mm diameter, otherwise it was described as 'negative' (Sokol, 1975).

Skin temperature was recorded over the centre of the developing lesion and at two control sites, 10 cm proximal and distal to the lesion. Temperature measurements were made with a non-contact infra-red radiation thermometer (Model KT41, Heinmann GmbH, Weisbaden-Dotzheim, FRG) which has a focal skin measuring area of 75 mm^2 and a temperature resolution of 0·05°.

Dermal blood flow was measured at the same sites using a laser-Doppler flowmeter (Model PF2, Perimed, Stockholm, Sweden): the output signal of this instrument (RBC flux) is related to the product of the number of moving red blood cells and their mean velocity within a small hemispherical volume of tissue extending approximately 1·5 mm into the dermis. Details of the machine settings and of the interpretation of the laser-Doppler signal have been recorded previously (Beck & Spence, 1986).

Intradermal pH was measured electrometrically from the potential difference between an internally insulated, 50 μm tip diameter, pH-sensitive electrode and an open-tipped agar/KCl reference pipette of similar diameter. Details of the construction and properties of the pH and reference electrodes and of the methods for signal recording and interpretation have been published previously (Harrison & Walker, 1977, 1979, 1980); only those technical details that are relevant to the present study are described here.

The pH electrodes were calibrated in two standard phosphate buffers (Corning Medical and Scientific, Halstead, Essex) against a reference pipette at 32° before each measurement. Standard buffer A had a pH of 6·838 at 37° and 6·850 at 32° and standard buffer B had values of 7·382 at 37° and 7·394 at 32°: the manufacturer quoted a standard deviation of $\pm$0·005 pH units for their products. A calomel half-cell was used to monitor the potential of the reference pipette. After completion of the pre-experimental calibration, the pH and reference electrodes were sterilized for 3 hr in Cidex (Arbrook Ltd, Livingstone, West Lothian). In order to minimize any possibility of cross-infection, the same pair of pH electrodes was used in a given subject for the whole series of measurements. Fresh reference electrodes were used for each measurement.

In order to insert the electrodes into the dermis, a small puncture was made in the epidermis with a 25-gauge disposable needle which was then removed and replaced with the relevant electrode. The two pH electrodes were always inserted at an angle of 30° so that their tips lay at a depth of about 2 mm within a 3 mm radius of the injection site (Fig. 1). From previous measurements of skin thickness at tuberculin skin test sites (Beck *et al.*, 1986b), it can be deduced that the tip of the pH micro-electrode lay within the region of the DHS reaction. The reference electrode was inserted some 30 or 40 mm distal to the pH electrodes. The electrodes were held in place at the correct angle using a small foam rubber cushion and adhesive tape.

A stabilization time of 10 min was allowed after insertion of the electrodes before reading the pH electrode voltage. By this time any local reactive hyperaemia had subsided and the electrode potentials were stable. The electrodes were recalibrated immediately after each measurement. If an electrode potential in either of the standard buffers differed by more than 5 mV from its pre-experimental value, then the recording from that electrode was rejected. Dermal pH values were then calculated from both the pre- and post-experimental calibrations and the mean value was taken.

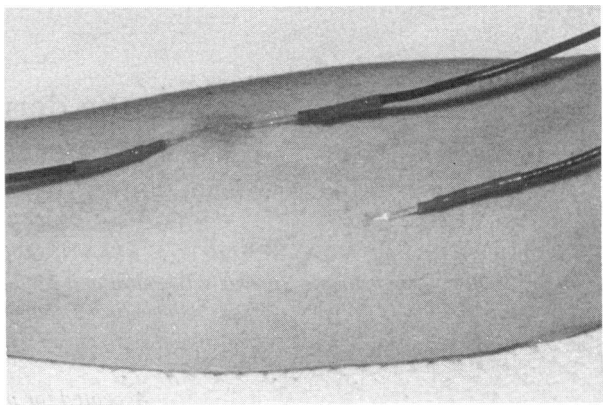

Figure 1. Photograph showing two glass micro-electrodes implanted into the dermis at the site of the tuberculin reaction and the reference electrode in the adjacent normal skin. This procedure allows the measurement of the pH of the dermis, but does not cause noticeable haemorrhage or other overt damage.

The performance of an electrode varies with temperature, but the isothermal characters of each electrode are unique. Accordingly, the dependence of electrode potential upon changes in temperature was determined for each electrode at the start of the study to allow correction for the differences in temperature between the skin site at the time of pH measurement and the calibration conditions. Harrison & Walker (1979) have shown that lowering skin temperature induces a rise in local pH; the results of measurements in the present study were corrected to a 'standard skin temperature' to allow valid comparison of the changes during the course of the tuberculin test reaction. Statistical analysis of changes in dermal pH during the course of the tuberculin delayed-type reaction was carried out using Student's *t*-test for paired values.

RESULTS

Visual, surface temperature and blood flow changes in the tuberculin skin test

The findings on the six subjects are summarized in Fig. 2. All but one of the subjects developed induration > 5 mm mean diameter at the site of antigen injection: in two subjects this feature was maximal at +48 hr and in individual subjects at +24, +72 and +96 hr. An erythematous response with mean diameter about twice that of the area of induration was seen in all the 'positive' subjects, and a smaller area of erythema (about 6 mm maximal diameter) was seen over the first 2 days in the negative reactor.

The extent of rise in skin temperature over the tuberculin test (ΔT) was calculated from the difference between the value recorded over the site of antigen injection and the mean of the values at the proximal and distal control sites. Review of the daily temperature measurements showed a positive ΔT in all five 'positive' subjects; it was greatest at +48 hr in two subjects and at +72 hr in the other three subjects. There was no significant rise in temperature in the 'negative' reaction, despite the small area of visible erythema.

The velocity of blood flow in the dermal microcirculation

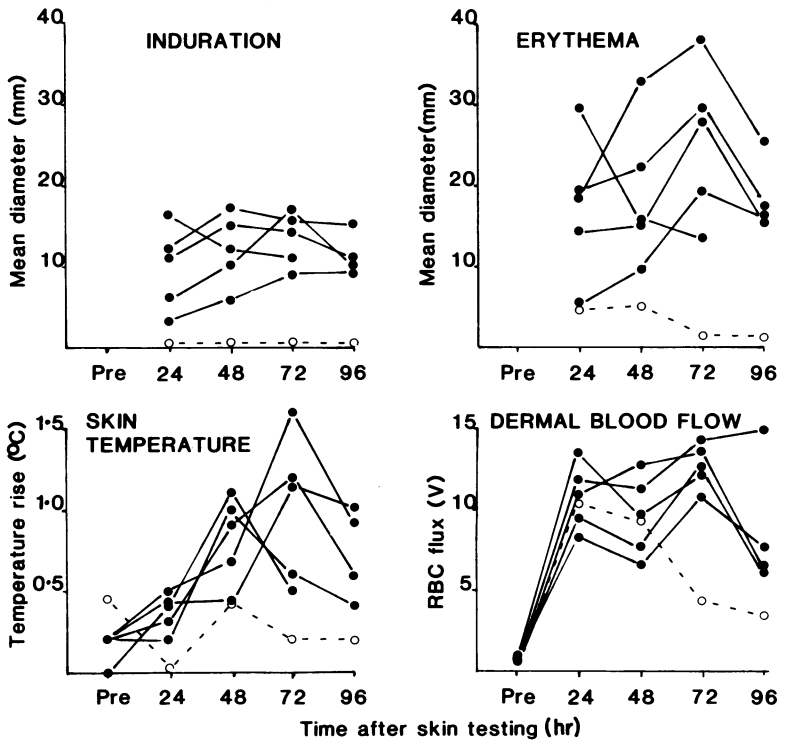

Figure 2. Sequential changes in erythema, induration, skin temperature and dermal blood flow (RBC flux). The subject who did not develop induration (open circles) showed relatively little rise in skin temperature, but had a change in RBCflux in laser-Doppler velocimetry (indicating an increased blood flow velocity) generally comparable to that seen in the other subjects (closed circles).

was raised over the antigen injection site in all subjects. The $RBCflux_{max}$ recorded at the centre of the lesion was 15·8 (SD 2·0, range 12·3–21·0) times greater than the signal recorded at the same skin site before testing. The maximal values were achieved at +24 hr in two subjects, at +72 hr in three subjects, and at +96 hr in the remaining subject. In the 'negative' reactor the $RBCflux_{max}$ signal peaked at +24 hr (15-fold increase over the pretest signal) and thereafter fell rapidly. The phenomenon of vasomotion (spontaneous rhythmical fluctuations in blood flow of relatively large amplitude and variable frequency) was particularly prominent at +72 hr and at +96 hr in all subjects, including the 'negative' reactor. These findings were similar to those reported in a previous investigation (Beck & Spence, 1986) and confirm that the 'positive' subjects are showing typical DHS reactions.

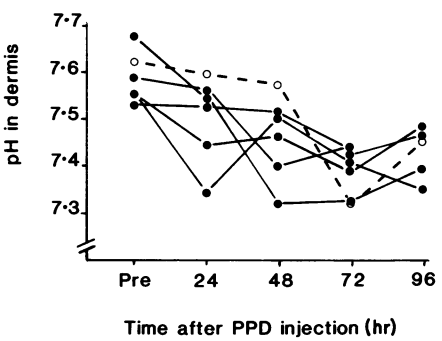

Figure 3. Changes in pH of the dermis during the course of the tuberculin reaction. The reduction in tissue pH occurs at the time when substantial numbers of lymphocytes and monocytes are infiltrating the dermis. The subject who did not develop induration (open circles) responded in a generally similar manner to those who showed induration (closed circles).

Measurements of dermal pH

The insertion of pairs of glass micro-electrodes into the dermis at the site of the tuberculin test did not cause haemorrhage or clinically apparent damage. Technically valid measurements were obtained at each session from all subjects: these are summarized in Fig. 3. Before skin testing, the measurements of dermal pH were similar in all subjects (mean pH 7·58; SD 0·05). All subjects showed a fall in dermal pH by 24 hr (mean fall of 0·10 pH units; SD 0·07) and this change was statistically significant ($t = 2·84$; $P < 0·05$). Thereafter, the measurements of dermal pH did not differ significantly from the +24 hr reading, although the general trend was for the lowest value to be recorded at +72 hr (mean fall of 0·20 pH units, SD 0·10 compared with the preinjection value; $t = 5·91$, $P < 0·002$) with some recovery by +96 hr (mean fall from start of experiment of 0·16 pH units, SD 0·08). For four 'positive' reactors the minimal value of dermal pH was found at the time when erythema/induration was most prominent, but in the other 'positive' reactor the pH minimum preceded the maximum clinical changes and, in the 'negative' reactor, a substantial fall in pH (0·29 units) was seen in non-indurated skin. The extent of local acidosis was also unrelated to the rise in skin temperature or changes in RBCflux.

DISCUSSION

After PPD skin testing, five out of six subjects developed an area of induration >5 mm diameter and so would be regarded as 'positive' reactors (Sokol, 1975); the remaining subject was negative on this conventional criterion, but nevertheless showed erythema from +24 to +48 hr localized to the antigen injection site. These are the expected appearances in young adults in the local community where BCG vaccination is widely practiced in childhood. The changes in cutaneous blood flow patterns measured by laser-Doppler velocimetry were typical of a DHS response to skin testing with tuberculin (Beck & Spence, 1986).

The 'negative' responder had an increased laser-Doppler signal comparable to that seen in the 'positive' reactors, but the skin showed little visible change in colour or consistency and no temperature increase. In our previous experiments (Beck & Spence, 1986) some of the subjects demonstrated greater temperature increases over the injection site and more florid erythema compared to our present subjects. It appears that the rise in skin temperature is related to the visible changes of erythema and we have previously suggested that both result from increased blood flow in the deeper plexuses of the dermis. By contrast, the increased laser-Doppler signal is less well related to skin colour and has probably arisen from accelerated flow in the more superficial plexuses and dermal papillary loops (Beck & Spence, 1986).

The present experiments showed that the dermal pH falls substantially during the response to tuberculin testing. Despite the individual variation, it is clear that the change is established by 24 hr. The preinjection control mean skin pH value of 7·58 (SD 0·07, $n=11$) compares well with the mean value of pH 7·54 (SD 0·09, $n=160$) measured in the lower legs and feet of 40 similarly aged subjects. The physiological reasons for this apparently high value of dermal pH have been discussed in a previous report (Harrison & Walker, 1979). The slightly higher mean value from the present series of experiments is not statistically significant, and could be explained by the different method used here for determining skin temperature. Review of the literature has shown that the occurrence of tissue acidosis at the site of skin testing has not been recorded previously.

Very many lymphocytes and monocyte/macrophages emigrate from the blood capillaries and accumulate at the site of antigen injection (Poulter *et al.*, 1982; Scheynius *et al.*, 1982); comparable cellular infiltrates can occur in some clinically 'negative' reactions (Beck *et al.*, 1986a). The great increase in cell numbers will by itself place a metabolic load on the tissue, with increased demand for oxygen and nutrients and the production of much more CO_2 and metabolites. Moreover, many of the infiltrating lymphocytes undergo activation (Platt *et al.*, 1983; Coghill *et al.*, 1985) and so the metabolic requirements of the individual cells will be expected to increase (Wang, Marquardt & Foker, 1976; Wang, Foker & Tsai, 1980). The great increase in blood flow rate (ranging from a 12·3× to a 21·0× increase) in the dermis will clearly compensate to some extent for the increased metabolic demand of the infiltrating cells, but the present study makes it clear that this hyperaemia is insufficient to prevent the development of a mild local acidosis, beyond the buffering power of the tissues. It was noticeable that the pH did not fall in the 'negative' reactor until the initial hyperaemia had passed off. It is not clear from the present experiments whether there is complete aerobic metabolism with acidosis resulting from an increase in tissue CO_2, or whether in the extreme conditions some anaerobic metabolism has occurred with resultant additional accumulation of lactate in the interstitial tissues.

Mild local acidosis is obviously not harmful in the skin test site because this reaction almost invariably resolves within a few days with no permanent deformity. However, in a chronic infection with ongoing type IV response, local acidosis may seem to be disadvantageous, but this may not necessarily be so since Mihm & Droge (1985) have produced preliminary evidence to indicate that high concentrations (10^{-2} M) of lactate may be advantageous to lymphocytes during activation, because cells at this stage have restricted capacity for energy production (Wang *et al.*, 1976, 1980). If this view prevails, then local acidosis may confer a small advantage by potentiating the earliest stage of the effector cell-mediated response.

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