*Immunology* 1976 **31** 665

# The use of 1-fluoro-2,4-dinitrobenzene as an affinity label for the antigen receptor of delayed hypersensitivity

H. P. GODFREY *Institute for Experimental Immunology, University of Copenhagen, Copenhagen, Denmark*

*Received* 5 *February* 1976; *accepted for publication* 11 *March* 1976

**Summary.** The passive transfer of delayed contact sensitivity to dinitrophenyl can be specifically inhibited by brief treatment of lymph node cells (LNC) from sensitized guinea-pigs with 1-fluoro-2,4-dinitrobenzene (DNFB). Analysis of KCl extracts of LNC previously treated with [$^3$H]DNFB, using polyacrylamide gel electrophoresis, revealed a peak of radioactivity present in both sensitive and normal cell extracts as well as a peak found only in sensitive cell extracts. Cell-free extracts appeared capable of transmitting delayed-onset skin reactivity to non-sensitized recipients.

## INTRODUCTION

The primary antigen–cell interaction in delayed hypersensitivity (DH) has been assumed to involve the specific interaction of antigenic determinants with receptor molecules located on the surface of the sensitized thymus-derived small lymphocyte (Humphrey, 1967; Schlossman, 1972). These antigen receptors appear to be synthesized by the cells themselves and not adsorbed from plasma or other tissue fluids since the 'classic' DH reaction (as opposed to other hypersensitivities of delayed onset) can be transferred passively only by viable sensitized lymphocytes and not by conventional serum antibodies (Bloom & Chase, 1967; Asherson and Zembala, 1970; Askenase, 1973).

Correspondence: Dr H. P. Godfrey, Department of Pathology, Health Sciences Center, School of Basic Health Sciences, S.U.N.Y., Stony Brook, New York 11794, U.S.A.

The physical nature of the antigen reactive molecule of DH is unknown. Although its specificity for antigen differs from conventional serum antibodies in several ways (Gell, 1944; Gell & Benacerraf, 1961; Schlossman, 1972; Benacerraf & Katz, 1974), it has been suggested that the DH antigen receptor may be composed of proteins antigenically related to the immunoglobulins (Greaves, 1970). Evidence both for (Greaves, 1970; Mason and Warner, 1970; Rajapakse, Papamichail and Holborow, 1973) and against (Crone, Koch & Simonsen, 1972; Koch & Nielsen, 1973; Godfrey, 1976b) this hypothesis has been presented in the past few years.

A different method for studying the combining site of the DH antigen receptor has employed an affinity labelling reagent, a method previously used to investigate the combining sites of soluble antibodies (Singer, 1967) and cell-bound antigen receptors of the passively transferred humoral response (Plotz, 1969). These reagents are bifunctional molecules structurally and chemically related to the antigen used to elicit the immune response which contain a chemically reactive group to provide a covalent link with amino acid residues in the binding site. Hill (1971) reported that $\varepsilon$-2-nitro-4-diazophenyl-L-lysine specifically blocked the passive transfer of DH to dinitrophenyl (DNP) in guinea-pigs and could be used as a trace label in the isolation of a 'delayed hypersensitivity antibody' from

extracts of sensitized cells. The isolated material was capable of transferring delayed-onset sensitivity to non-immunized recipients (Hill & Nissen, 1971).

Since the passive transfer of DH in guinea-pigs by cell-free extracts remains controversial (Bloom & Chase, 1967), it was important to attempt to reproduce Hill's findings with a less complex affinity label if possible. The ease of handling and ready availability of 1-fluoro-2,4-dinitrobenzene (DNFB) and of high specific activity [$^3$H]DNFB suggested the use of this compound. As the lymph node cells (LNC) of guinea-pigs sensitized with 1-thiocyano-2,4-dinitrobenzene (DNTB) in Freund's complete adjuvant (FCA) have recently been shown by *in vitro* assays for DH (Godfrey, 1976a) and by cell chromatography (Godfrey & Gell, 1976) to have hapten-specific receptors for DNP they appeared to be particularly suitable for this study. The results of these investigations indicate that DNFB can be used as an affinity label for the antigen receptor(s) of DH.

## MATERIALS AND METHODS

*Animals*

Male Hartley guinea-pigs, $350 \pm 50$ g at the time of sensitization were obtained from OLAC Northern, Red Hill Farm, Birmingham or Statens Serum Institut, Copenhagen (Denmark) (Godfrey, 1976a).

*Sensitization*

Guinea-pigs were sensitized with 1 mg DNTB (K + K Laboratories, Plainview, New York) in 1 ml FCA (Difco) containing 1 mg mixed strains of heat-killed human *Mycobacterium tuberculosis* (Weybridge) injected in multiple sites and skin tested as previously described (Godfrey & Baer, 1971). Non-injected animals obtained at the same time as sensitized guinea-pigs were used as normal (non-immunized) controls.

*Chemicals and reagents*

DNFB (Eastman Organic Chemicals, Rochester, New York) and chromatographically pure DNP–glycine (BDH, Poole, Dorset) were dissolved in phosphate-buffered saline (PBS), pH 7·2 containing 3 mM KCl or in 100 mM $Na_2HPO_4$, respectively, as previously described (Godfrey, 1976a). BSA (bovine serum albumin) (Armour Pharmaceuticals), PPD (Statens Serum Institut, Copenhagen) and DNP–BSA which contained thirty-seven DNP groups per mole conjugate were stored as sterile solutions in PBS. 1′-Hydroxy-3-n-pentadecylcatechol (HPDC) was a gift from Dr H. Baer. Other chemicals used were of reagent grade.

Aliquots of high specific activity [$^3$H]DNFB in benzene (> 10000 mCi/mmole) obtained from the Radiochemical Centre, Amersham, were evaporated to dryness in glass vials and suitable volumes of PBS solutions of DNFB, 5 mM (1 mg/ml) added to give a specific activity of 200–2000 mCi/mmole. Solutions of DNFB were stored at 4° and discarded after 72 h.

*Tissue culture medium*

RPMI-1640 containing 15 mM Hepes (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid), pH 7·2, 0·085% $NaHCO_3$ (Biocult, Glasgow) and supplemented with antibiotics, glutamine and on occasion, 10 per cent (v/v) heat-inactivated foetal calf serum (FCS) was used throughout.

*Preparation and treatment of LNC*

Lymph nodes were aseptically removed from exsanguinated, sensitized (and control normal) guinea-pigs 14 days (cell transfer experiments) or 60 days (cell extract experiments) after sensitization and LNC prepared by a standard method (Oppenheim, Wolstencroft & Gell, 1967). The LNC were washed twice, counted, and viability determined by trypan blue dye exclusion. Viability was greater than 85 per cent. DNFB, [$^3$H]DNFB or an equal volume of PBS was added to a final concentration of 50 μM (10 μg/ml) to LNC suspensions adjusted to contain $10^8$ cells/ml in PBS and the suspensions incubated at 37° for 45 min without agitation. In some experiments, LNC were incubated in DNP–BSA (final concentration, 30 μg/ml) or DNP–glycine (final concentration 1 mM). After incubation, the cells were washed four times at 4° with RPMI-1640 (cell extracts) or 1640-FCS (cell transfers), counted and viability determined. For cell transfers, LNC were suspended to $2 \times 10^8$ LNC/ml; each recipient received $6 \times 10^8$ viable cells or the same volume 1640-FCS intraperitoneally. In those experiments where recipients received a mixture of DNFB-treated and untreated LNC at the same time, the cells were mixed immediately before injection; at least $5 \times 10^8$ viable cells were injected. Donor: recipient ratios were 2:1. Recipients were skin tested 18 h after injection. Cell extracts were prepared using KCl as described by Reisfeld, Pellegrino & Kahan (1971) by adding solid KCl to a final concentration of 3 M to LNC suspensions ($5 \times 10^7$

cells/ml) and shaking vigorously at room temperature to dissolve the KCl. KCl has been shown to solubilize membrane proteins directly and without involving cellular proteases (Oh, Pellegrino & Reisfeld, 1974). Extracts were dialysed against large volumes of PBS using Visking dialysis membranes and concentrated by Amicon pressure dialysis using UM10 membranes. They were used as soon as possible after preparation, either in polyacrylamide gel electrophoresis or for intraperitoneal injection into non-immunized recipient guinea pigs. Extracts were injected at a donor:recipient ratio of 3:1.

Radioactivity of [$^3$H]DNFB and [$^3$H]DNP (cell-bound and in extracts) was counted in a Phillips scintillation counter using a PPO-POPOP-Triton X-100-toluene counting fluid and was converted to d.p.m. by the channels-ratio method with external standardization. Protein content of extracts was determined by the Folin–Wu phenol technique (Lowry, Rosebrough, Faw and Randall, 1951).

*Skin tests*

Testing for contact sensitivity and for reactions to intracutaneously injected materials was performed as previously described (Godfrey & Baer, 1971; Godfrey, 1976a). Skin test sites were observed hourly for the first 4 h, at 16–18 h and at 24 h. Any early erythema and oedema was noted qualitatively. Delayed contact reactions are reported as geometric mean threshold response at 24 h; for purposes of computation, animals not responding to the highest tested dose were assumed to react at a 3-fold larger dose (Godfrey & Baer, 1971). Delayed-onset erythema reactions are reported as geometric mean reaction size at 24 h as estimated from the product of measurements of erythema in two perpendicular directions; for purposes of computation, reactions of 0 were assigned a value of 1. The results of skin tests were evaluated by analysis of variance and Student's *t*-test.

*Cell chromatography and assay of cellular immunity*

Cell chromatography over DNP-BSA polyacrylamide beads was performed as described (Godfrey & Gell, 1974) using LNC from sensitive and normal guinea-pigs. LNC populations before ('B') and after ('A') chromatography were examined for their ability to elaborate macrophage agglutinating factor (MAF) in response to exposure to DNFB and PPD (Godfrey, 1976a) and for their ability to rosette with DNP–sheep red cells (SRBC) and SRBC (Godfrey & Gell, 1976). Indirect MAF titres are reported as the inverse of the last dilution of MAF to show a positive agglutination response. Rosette-forming cells (RFC) with DNP–SRBC and SRBC are reported per $10^6$ LNC. Aliquots of B and A cell populations were reacted with [$^3$H]DNFB and cell-bound radioactivity determined as described above.

*Polyacrylamide gel electrophoresis*

Sera and LNC extracts from sensitive and normal guinea-pigs were analysed by Mrs P. Crockson, Department of Experimental Pathology, University of Birmingham Medical School (England) using a Shandon disc-gel apparatus (Shandon Southern Instruments, London) and the following procedure. The sample containing approximately 200 $\mu$g protein and phenol red as solvent-front indicator was run into the stacking gel (3·5 per cent gel, pH 6·9) at 2·5 mA until the solvent front reached the interface between stacking and running gels. The electrophoresis was continued at 5 mA (7 per cent gel, pH 8·9) until the solvent front was 1 cm from the end of the tube. The tank buffer was 0·25 M Tris (Sigma Chemical Company, St Louis, Missouri)-glycine buffer, pH 8·3; 0·12 M Tris–phosphate, pH 6·9 and 0·12 M Tris–HCl buffers were the other buffers used. After electrophoresis, the gels were removed from the tubes, cut off at the solvent front and either sliced into 1 mm pieces and slices eluted with PBS for counting of radioactivity or stained with 1 per cent naphthalene black in 7 per cent acetic acid for 1 h and destained electrolytically.

## RESULTS

### Specific inhibition of passive transfer of DH

Passive transfer of a delayed response to dinitrophenyl by guinea-pig LNC was significantly inhibited by a brief treatment of LNC with DNFB without affecting the transfer of delayed-onset reactivity to PPD (Table 1). Treatment of the cells with DNP–glycine or DNP–BSA did not inhibit passive transfer of delayed reactivity to DNP; mixtures of DNFB-treated and untreated LNC transferred delayed reactivity to both antigens about as well as untreated cells did with no disproportionate decrease in the response to DNTB. No responses were transferred by injections of normal cells or media.

**Table 1.** Effect of treatment of guinea pig lymph node cells with DNP-containing materials on the subsequent passive transfer of delayed hypersensitivity

| | Number of animals with threshold skin reactivity to: | | | | | | Mean threshold reaction (nmoles) | | Mean erythema ($mm^2$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DNTB (nmoles) | | | | HPDC (nmoles) | | DNTB | HPDC | BSA (30 $\mu$g) | PPD (30 $\mu$g) |
| Sensitive cells transferred: | n.r. | 1500 | 1000 | 500 | n.r. | 50 | | | | |
| Treatment | | | | | | | | | | |
| None | 1 | 7 | 6 | 2 | 8 | 0 | 1210* | 150 | 2·45 | 41·2* |
| DFNB (10 $\mu$g/ml) ($50\ \mu$M) | 4 | 2 | 0 | 2 | 8 | 0 | 1980 | 150 | 2·00 | 48·3* |
| DNP–glycine ($1000\ \mu$M) | 0 | 2 | 3 | 0 | 5 | 0 | 1180* | 150 | 2·35 | 30·3* |
| DNP–BSA ($30\ \mu$ gM/ml) ($15\ \mu$M DNP) | 0 | 1 | 4 | 0 | 5 | 0 | 1080* | 150 | 9·64 | 29·0* |
| None + DNFB-treated mixed at 3:1 ratio | 1 | 6 | 2 | 0 | 7 | 2 | 1550* | 121 | 3·05 | 25·0* |
| *Controls* | | | | | | | | | | |
| Normal cells transferred | 4 | 1 | 1 | 0 | 6 | 0 | 2910 | 150 | 6·04 | 3·03 |
| No cells transferred | 18 | 8 | 1 | 0 | 15 | 0 | 3070 | 150 | 2·74 | 4·20 |

n.r. = Not reactive to highest tested dose.

* Significantly different from controls, $P<0·05$.

### Labelling of sensitized and normal LNC by [$^3$H]DNFB

The specific inhibition by DNFB of the passive transfer of DH to DNP suggested that DNFB was being bound by LNC. If DNFB was acting as an affinity label of antigen receptors of LNC responsible for passive transfer of DH, it might be possible to isolate these labelled receptors from cell extracts using [$^3$H]DNP as a trace label. Sensitive and normal LNC were reacted with [$^3$H]DNFB and extensively washed; in one experiment there were 19,900 d.p.m./$10^7$ sensitive LNC and 10,600 d.p.m./$10^7$ normal LNC, nearly twice as much radioactivity on the sensitive LNC. Sensitive LNC bound 65 per cent more [$^3$H]DNFB than normal LNC (mean of five experiments), a difference significant at the 2 per cent level (*t*-test).

To investigate whether the bulk of this radioactivity was bound by antigen receptors, sensitive and normal LNC were chromatographed on DNP polyacrylamide beads and the ability of LNC to bind [$^3$H]DNFB before and after chromatography compared. Cell chromatography removed cells which mediated MAF production to DNFB and formed rosettes with DNP–SRBC without affecting those cells responding to PPD or forming rosettes with SRBC (Table 2). On the other hand, changes in cell-bound [$^3$H]DNP correlated better with the percent LNC recovered after chromatography than with the assays of cellular immunity suggesting that much of the radioactivity was not associated specifically with DH antigen receptors.

### Characterization of LNC extracts

Even though the bulk of [$^3$H]DNP did not appear to be specifically associated with DH antigen receptors, extracts of LNC were prepared. KCl extracts of sensitive and normal LNC treated with [$^3$H]DNFB before extraction had 280 nm/260 nm absorbance ratios between 1·1 and 1·2, ratios consistent with the presence of nucleic acids as well as proteins in these extracts. The specific activity of extracts (d.p.m./mg protein estimated chemically) from sensitive LNC was $2{\cdot}04 \pm 0{\cdot}88$ times that of extracts from normal LNC (mean of four experiments ± standard error). Stained polyacrylamide gels of electrophoresis of extracts and sera are shown in Fig. 1. Unstained gels of extracts from sensitive and normal LNC treated with [$^3$H]DNFB had a sharply defined yellow band (Fig. 1, peak 1) at $R_f = 0{\cdot}95$ and a broad brownish band (Fig. 1, peak 2) at about $R_f = 0{\cdot}60$. Peak 1 was heavily stained for protein, but peak 2 was not readily discernible after staining. Peak 2 appeared to migrate faster than albumin. Extracts from both sensitive and normal LNC not treated with [$^3$H]-DNFB showed only peak 2 in unstained gels, but peak 1 was visible in all cases after staining.

If the gels from electrophoresis of [$^3$H]DNFB treated sensitive and normal LNC extracts were eluted in PBS rather than stained for protein, the results shown in Fig. 2 were obtained. Extracts from sensitive and normal LNC had a peak of radioactivity at $R_f = 0{\cdot}95$ coincident with peak 1 of the unstained gel. Sensitive extracts had in addition a second peak of radioactivity at $R_f = 0{\cdot}54$, some-

**Table 2.** Effect of cell chromatography on [$^3$H]DNFB bound to guinea-pig LNC

| Expt no. | Donor sensitized | Percentage of cells recovered after chromatography | Percentage change cell-bound [$^3$H]DNP after chromatography | MAF titre* | | | | Rosettes/$10^6$ LNC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | DNFB | | PPD | | DNP-SRBC | | SRBC | |
| | | | | B | A | B | A | B | A | B | A |
| 1 | Yes | 99 | −15 | 1000 | <3 | 100 | 1000 | 120 | 40 | | |
| 2 | Yes | 70 | −3 | 1000 | <3 | 1000 | 100 | 240 | 80 | | |
| 3 | Yes | 117 | +38 | | | | | 408 | 97 | 42 | 44 |
| 4 | Yes | 98 | +0·6 | | | | | 136 | 84 | 520 | 344 |
| 1 | No | 117 | +13 | <3 | 3 | <3 | <3 | | | | |
| 2 | No | 91 | 0·0 | | | | | 240 | 80 | | |
| 3 | No | 68 | −28 | | | | | 98 | 64 | 166 | 156 |
| 4 | No | 94 | +49 | | | | | 164 | 92 | | |

See Materials and Methods for details.

* B = before chromatography; A = after chromatography.

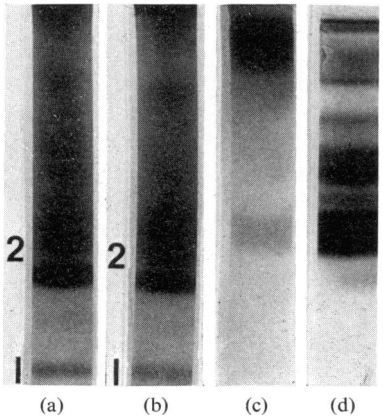


**Figure 1.** Naphthalene black-stained polyacrylamide gels of electrophoresis of KCl extracts of (a) sensitive and (b) normal LNC. 1 and 2 indicate the position of bands seen in unstained gels. Electrophoresis of sera from animals donating cells is shown in (c) and (d). (c) was applied at 100-fold lower concentration than (d). The origins of all gels have been aligned and the gels cut off at the solvent front. See text for details.

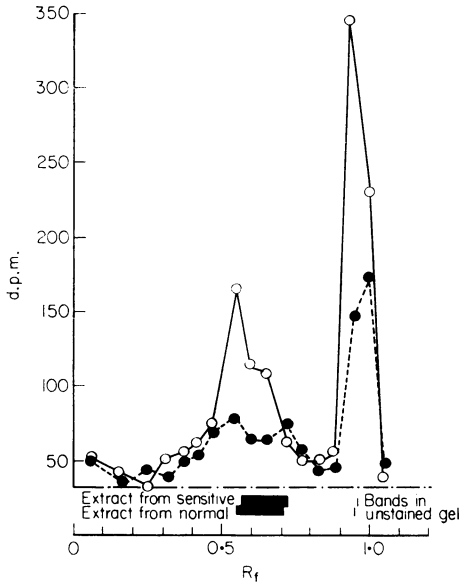


**Figure 2.** Polyacrylamide gel electrophoresis of KCl extracts of [$^3$H]DNFB-treated LNC. Pools of slices from six parallel gels eluted with PBS and counted for each point. Below is shown position of bands seen in unstained gels. See the Materials and Methods section for details.

what slower than peak 2 of the unstained gel. Peaks as described were present in four out of four extracts of sensitive and normal LNC examined. In 4 experiments, the mean ratio of radioactivity in the two peaks (sensitive/normal ± standard error) was 2·6 ± 0·6 for the faster peak and 5·4 ± 1·5 for the slower peak.

### Activity of LNC extracts

Unfractionated extracts of sensitized and normal LNC were injected into normal guinea-pigs and recipients skin tested 18 h later (Table 3). Extracts from sensitized LNC not treated with DNFB transferred statistically significant delayed-onset sensitivity to both antigens. No delayed-onset reactivity was transferred by extracts of normal LNC. While the numbers of animals examined were too small to be statistically significant, there was a trend which suggested that extracts from sensitized LNC treated with DNFB transferred delayed-onset erythema to PPD but not to DNTB. Histological examination of the skin reactions was technically unsatisfactory.

## DISCUSSION

The foregoing data suggest that DNFB can be used as an affinity label to specifically inhibit the passive transfer of delayed-onset reactions to DNP and would appear to confirm the report of Hill (1971). Specific inhibition of the passively transferred delayed response required the use of a chemically reactive molecule since neither DNP–glycine nor DNP–BSA were effective in inhibition of DH. Incidentally, incubation of LNC with protein antigens has previously been reported not to interfere with subsequent passive transfer of DH by Bloom, Hamilton & Chase (1963). Since mixtures of DNFB-treated and untreated LNC transferred DH to DNP nearly as well as untreated sensitive LNC alone, it is unlikely that the specific inhibition caused by DNFB was due to mere modification of LNC surfaces rather than to true affinity labelling of specific antigen receptors (Naor, Mishell & Wofsy, 1970).

Although sensitive LNC repeatedly bound more labelled DNFB than nonsensitive LNC, the amounts bound were not markedly reduced by chromatographing the cells over DNP–polyacrylamide beads, implying non-specific binding of the majority of cell-associated DNP. However, electrophoresis of extracts of the dinitrophenylated LNC revealed a distinct difference in the distribution of radioactivity

**Table 3.** Passive transfer of delayed-onset reactions by cell-free LNC extracts

| Donor status | LNC reacted with DNFB | Number of animals with threshold skin reactions to: DNTB (nmoles) n.r.* | 1500 | 1000 | 500 | 250 | 100 | HPDC (nmoles) n.r. | 50 | Mean threshold reaction (nmoles) DNTB | HPDC | Mean erythema ($mm^2$) BSA (30 $\mu$g) | PPD (30 $\mu$g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sensitized | No | 0 | 0 | 0 | 2 | 2 | 1 | 5 | 0 | 275† | 150 | 2·30 | 26·0† (4; 30; 30; 30; 110)‡ |
| Sensitized | Yes | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 4500 | 150 | 1·42 | 86·9 (42; 180)‡ |
| Not sensitized | No | 4 | 1 | 0 | 0 | 0 | 0 | 4 | 1 | 3610 | 120 | 5·40 | 5·04 |
| Not sensitized | Yes | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 4500 | 150 | 2·52 | 1·82 |
| PBS injected | | 12 | 1 | 0 | 0 | 0 | 0 | 6 | 0 | 4140 | 150 | 2·64 | 3·39 |

Extracts prepared from LNC from three donors injected intraperitoneally into one recipient 18 h before skin testing. See the Materials and Methods section for further details. Delayed-onset reactions not present at 0–4 h and maximal at 24 h except for the one reading underlined, in which swelling and erythema was present at 4 h.

* Not responsive to highest tested dose.

† Significantly different from controls, $P<0·05$.

‡ Individual reaction size in parentheses.

in sensitive and normal cells as well as preferential labelling of molecules of these cells. Extracts of DNFB-treated sensitive and non-sensitive cells contained a fast moving radioactive peak which stained for protein and which was yellow in unstained gels; this material presumably is a DNP–protein conjugate. Since a rapidly migrating protein was present in extracts from LNC not treated with DNFB (suggesting that the rapid mobility of the DNP conjugate was not a result of dinitrophenylation), this protein might be a candidate for the autologous 'carrier protein' (Bloom & Chase, 1967) to which reactive sensitizers have been postulated to bind in the course of becoming complete immunogens. A second more slowly migrating peak of [$^3$H]DNP was found only in extracts of sensitive LNC and may represent an antigen receptor. Neither of the radioactive peaks represent native IgG, as molecules of this size did not migrate into the gel. The faster peak of radioactivity may represent a smaller molecule than the slower peak which in turn was slower than the haemoproteins in the extract. These haemoproteins may not be identical with hemoglobin (Geczy, Friedrich and de Weck, 1975). The method of preparation ensured that both peaks of radioactivity were non-dialysable molecules. These results also confirm Hill's report (1971).

Delayed-onset reactivity to DNP and PPD was transferred passively by cell-free extracts of sensitive LNC; extracts of DNFB-treated sensitive LNC transferred only reactivity to PPD. This aspect of the present study was also consistent with results reported by Hill & Nissen (1971). Because of technical difficulties encountered in examining these deayled-onset reactions histologically, it was not possible to confirm the mononuclear nature of any cellular infiltrates. Their aetiology remains obscure and is currently under study.

## ACKNOWLEDGMENTS

I wish to thank J. A. Lowe, Mrs P. Crockson and Hanne Nors for technical assistance and Professor P. G. H. Gell, Department of Experimental Pathology, University of Birmingham Medical School in whose department this study was begun under the tenure of a Moseley Travelling Fellowship, Harvard University and has been supported by grants from the Anna Fuller Fund, Danish Medical Research Council and Mary Rosenkjaers Legat.

## REFERENCES

Asherson G.L. & Zembala M. (1970) Contact sensitivity in the mouse. IV. The role of lymphocytes and macrophages in passive transfer and the mechanism of their interaction. *J. exp. Med.* **132**, 1.

Askenase P.W. (1973) Cutaneous basophil hypersensitivity in contact-sensitized guinea pigs. I. Transfer with immune serum. *J. exp. Med.* **138**, 1144.

Benacerraf B. & Katz D.H. (1974) Failure to induce tolerance to 2, 4-dinitrochlorobenzene contact sensitivity with a 2, 4-dinitrophenyl (DNP) conjugate of a copolymer of D-glutamic acid and D-lysine, a specific tolerogen for DNP B cells. *J. Immunol.* **112**, 1158.

Bloom B.R. & Chase M.W. (1967) Transfer of delayed-type hypersensitivity. A critical review and experimental study in the guinea pig. *Progr. Allergy*, **10**, 151.

Bloom B.R., Hamilton L.D. & Chase M.W. (1963) Effects of mitomycin C on the cellular transfer of delayed-type hypersensitivity in the guinea pig. *Nature (Lond.)*, **201**, 689.

Crone M., Koch C. & Simonsen M. (1972) The elusive T cell receptor. *Transplant. Rev.* **10**, 36.

Geczy C.L., Friedrich W. & Weck A.C. de (1975) Production and *in vivo* effect of antibodies against guinea pig lymphokines. *Cell. Immunol.* **19**, 65.

Gell P.G.H. (1944) Sensitization to 'tetryl'. *Brit. J. exp. Path.* **25**, 174.

Gell P.G.H. & Benacerraf B. (1961) Delayed hypersensitivity to simple protein antigens. *Advanc. Immunol.* **1**, 319.

Gell P.G.H. & Godfrey H.P. (1974) Studies on cell separations in delayed hypersensitivity. *Monogr. Allergy*, **8**, 89.

Godfrey H.P. (1976a) Hapten-specific responses to contact sensitizers: use of fluorodinitrobenzene to elicit migration inhibition and macrophage agglutination factors from lymph node cells of contact sensitive guinea pigs. *Immunology* **30**, 685.

Godfrey H.P. (1976b) Differences in sensitivity to a cytoxic antithymus-derived lymphocyte serum of cells mediating delayed-onset reactions in guinea pigs to hapten-protein conjugates and contactants. *Cell. Immunol.* **22**, 28.

Godfrey H.P. & Baer H. (1971) The effect of physical and chemical properties of the sensitizing substance on the induction and elicitation of delayed contact sensitivity. *J. Immunol.* **106**, 431.

Godfrey H.P. & Gell P.G.H. (1976) Separation by column chromatography of cells active in delayed-onset hypersensitivities. *Immunology* **30**, 695.

Greaves M.F. (1970) Biological effects of anti-immunoglobulins: evidence for immunoglobulin receptors on 'T' and 'B' lymphocytes. *Transplant. Rev.* **5**, 45.

Hill W.C. (1971) The antigen receptor in delayed-type hypersensitivity. I. Isolation with an affinity label. *J. Immunol.* **106**, 414.

Hill W.C. & Nissen B. (1971) The antigen receptor in delayed-type hypersensitivity. II. Passive sensitization of guinea pigs. *J. Immunol.* **106**, 421.

Humphrey J.H. (1967) Cell-mediated immunity-general perspectives. *Brit. Med. Bull.* **23**, 93.

Koch C. & Nielsen H.E. (1973) Effect of anti-light chain antibodies on rat leukocytes *in vitro*. *Scand. J. Immunol.* **2**, 1.

LOWRY O.H., ROSEBROUGH N.J., FAW L.A. & RANDALL R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265.

MASON S. & WARNER N.J. (1970) The immunoglobulin nature of the antigen recognition site on cells mediating transplantation immunity and delayed hypersensitivity. *J. Immunol.* **104,** 762.

NAOR D., MISHELL R.I. & WOFSY L. (1970) Specific inhibition of an anti-hapten response by chemical modification of cells. *J. Immunol.* **105,** 1322.

OH S.K., PELLEGRINO M.A. & REISFELD R.A. (1974) Hypertonic salt extraction of HL-A antigens: assessment of protease activity. *Proc. Soc. exp. Biol. (N.Y.)* **145,** 1272.

OPPENHEIM J.J., WOLSTENCROFT R.A. & GELL P.G.H. (1967) Delayed hypersensitivity in the guinea pig to a protein hapten conjugate and its relationship to *in vivo* transformation of lymph node, spleen, thymus and peripheral blood lymphocytes. *Immunology,* **12,** 89.

PLOTZ P.H. (1968) Specific inhibition of an antibody response by affinity labelling. *Nature (Lond.),* **223,** 1373.

RAJAPAKSE D.A., PAPAMICHAIL M. & HOLBOROW E.J. (1973) Immunoglobulin nature of PPD receptors on human T lymphocytes. *Nature: New Biol.* **245,** 155.

REISFELD R.A., PELLEGRINO M.A. & KAHAN B.D. (1971) Salt extraction of soluble HL-A antigens. *Science,* **172,** 1134.

SCHLOSSMAN S.F. (1972) Antigen recognition: the specificity of T cells involved in the cellular immune response. *Transplant Rev.* **10,** 97.

SINGER S.J. (1967) Covalent labelling of active sites. *Advanc. Prot. Chem.* **22,** 1.