

BRIEF NOTE

THE DISTRIBUTION OF DNP GROUPS IN LYMPHOID
SYSTEM OF RABBITS FOLLOWING
SKIN PAINTING WITH DNCB

2,4-Dinitrophenyl (DNP) groups have been found attached mainly to lymphocytes throughout the peripheral lymphoid system of guinea pigs following painting the skin with 2,4-dinitrochlorobenzene (DNCB) by the immunofluorescent method using the antibody against DNP groups¹⁾. Evidence has been shown which indicates that DNCB penetrates through the skin and bind chemically with the cell membrane of the cells in the lymphoid system²⁾. An analysis of the number of the 2,4-dinitrophenylated (DNP) cells in the lymphoid system at different time intervals after painting has showed that the incidence of such cells is high particularly in the lymph nodes draining the site of such painting with DNCB, and that it is maximal at 12 hours and declined quickly after that^{1,2)}. We should like to report such DNP cells are also found in the peripheral lymphoid system of rabbits.

Rabbits weighing 3 to 4 kg were painted with a total of 1 ml of a 5 per cent DNCB-ethanol solution on the shaved areas of both sides of foot pad skin. The popliteal lymph nodes were removed and cell suspension was prepared by teasing the nodes in PBS (0.01 M phosphate buffer saline, pH 7.2). DNP cells were detected by the immunofluorescent method using fluorescein isothiocyanate labelled antibody to DNP groups as described previously^{1,2)}. The percentage of the stained cells was determined by examination of the microscopic field in fluorescent light and conventional light alternately. Figure shows the incidences of DNP cells in the draining lymph nodes at various time intervals after painting the skin with DNCB. The percentage was maximal at 12 hours. The similar time courses have also been obtained in the experiments using guinea pigs and mice¹⁻³⁾.

For collecting lymph cells from the afferent lymphatics carrying lymph from the skin of the distal parts of the limbs, an operative approach was adopted from Soeberg et al.⁴⁾ Under anesthesia by an injection of sodium pentobarbital, intravenous infusions of saline were given at the rate of 250 ml/hour. The rabbit was placed on its back and a superficial incision was made in the skin of hind leg at right angles to the long axis on which DNCB was to be applied. A lymphatic which ran parallel with vena saphena medialis was identified using a binocular microscope and cannulae inserted into the distal

part of the vessel and secured in position by two sutures. The drainage area was then painted with 0.5 ml of 5 per cent DNCB-ethanol solution and lymph collection was carried out for two hours. The lymph cells were separated from the lymph by centrifugation and washed twice in PBS. DNP cells were also detected by the immunofluorescent technique as described above. Approximately 10 per cent of the cells were found to be stained. This suggests that a part of DNP cells detectable in the draining lymph nodes are formed in skin or in afferent lymphatics.

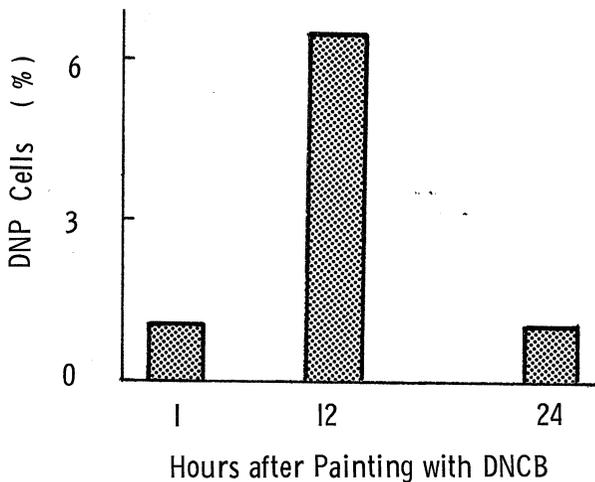


Figure. Frequencies of dinitrophenylated (DNP) cells which were obtained from the draining lymph nodes at various times after painting the foot pad skin of rabbits with 5% DNCB-ethanol solution.

The lymph collection was made at 12 or 24 hours after the drainage area was applied with DNCB. The lymph plasma was separated from the lymph by centrifugation. The lymph node cells which had been obtained from the intact rabbits, were incubated at 37°C for 10 minutes (5×10^6 /ml) in the lymph plasma. The cells were then washed three times with PBS and examined by the immunofluorescent method. DNP groups were detected on approximately 0.5 per cent cells. This result shows that unreacted DNCB is present in the afferent lymph at least for 24 hours after painting the skin with DNCB. It has also been demonstrated that unreacted DNCB remains in thoracic duct lymph of guinea pigs at least for 12 hours after painting the skin with DNCB.

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