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A Combined Regimen of Etretinate and UVB Modulate T6$^+$ and HLA-DR$^+$ Epidermal Cells

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Using anti-T6 and anti-HLA-DR monoclonal antibodies, this study was designed to attain what would do to epidermal Langerhans cell (LC) markers in psoriatic patients when two remedies, etretinate and UVB, having controversial effects on LC were put to use simultaneously. In normal and psoriatic subjects, HLA-DR$^+$ LC was approximately 80% of T6$^+$ LC and a single dose of UVB equivalent to minimal erythema dose (MED), reduced LC membrane markers to approximately 30% of non-irradiated control. The recovery of LC membrane markers, after a single dose of UVB exposure were significantly faster in the group of etretinate treated psoriatic subject than only UVB irradiated psoriatic control. Taken together, seemed to exert prompt recovery of normalization of the number of LC from the depletion following the UVB.

Key Words: *T6$^+$ and HLA-DR$^+$ epidermal cells, Aromatic retinoid, UVB.*

INTRODUCTION

Keratinocytes, melanocytes and suprabasal dendritic Langerhans Cells (LC) are the 3 populations of cell present in the mammalian epidermis. LC, 2 to 8 per cent of epidermal cell populations, are derived from the bone marrow and normally undergo gradual turnover. At the light microscopic level following histochemical staining for membrane ATPase, LC appear as a highly dendritic suprabasal epidermal cell. Ultrastructurally, LC are identified by their possession of a distinctive intracytoplasmic organelle known as the Langerhans cell granule (Birbeck granule; Birbeck et al., 1961). The cells that have the ultrastructural features of LC but lack the characteristic granule are termed indeterminate cells (thought to be LC precursor).

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In normal mammalian epidermis, LC and indeterminate cells are the only cells that express immune response-associated (Ia, HLA-DR) antigens and they also express a cell membrane antigen revealed by the monoclonal antibody (Mcab) OKT6 (Fithian et al., 1981). However, a small pupulation of indeterminate cells, lacking the Birbeck granule, is also labeled with OKT6 (Chu et al., 1982).

Both the T6 and HLA-DR Mcab used to delineate the LC populations do not label identical dendritic epidermal cell populations, either in normal or diseased skin (Mackie & Turbitt, 1983). Although the T6 reactivity is more sensitive than Ia reactivity in defining the LC (Harrist et al., 1983), HLA-DR antigen appears to be essential to cell interactions in immune response.

In the past few years, a great deal has been learned about the modulation of LC in the skin. Indeed, irradiation of mouse skin with ultraviolet B (UVB) leads to a reduction of the LC population, as reveald by the ATPase technique (Bergstresser et al., 1980; Aberer et al., 1981; Lynch et al., 1981). In addition, PUVA (psoralen UVA irradiation) (Strauss et al., 1980; Friedmann, 1981), glucocor-

ticosteroids application (Berman et al., 1983b), tape stripping of the epidermis (Streilein et al., 1982) and chemical carcinogen application on the skin (Muller et al., 1985) have reported to depletion of epidermal LC.

On the contrary, etretinate, one of the semi-synthetic derivative of vitamine A (Fernandez-Bussy et al., 1983; Haftek et al., 1983) and monobenzyl ether of hydroquinone (Rheins & Nordlund, 1986) have shown to increase the number of LC in human skin.

Lately, the combined regimen of etretinate and ultraviolet radiation (UVR) has been employed as one of the popular methods for the treatment of psoriasis. Multiple reports indicated that etretinate, in moderate dosages, notably enhances the effectiveness of other more routine antipsoriatic modalities such as PUVA, UVB, anthralin, and topical steroids(Voorhees & Orfanos, 1981). Moreover, in combination with these conventional agents, a lower retinoid dose as an adjuvant treatment and a reduced dose of the conventional agent were successfully used in most cases.

This study was conducted to determine, using T6 and HLA-DR Mcab what these combined treatment agents of etretinate and VUB would do to the density of the epidermal LC.

MATERIALS AND METHODS

Materials

Sixteen patients with chronic psoriatic plaques of moderate severity were taken for this study. They were eight men and eight women aged between 20 to 52, and divided into two groups considering their ages. One group was irradiated with a single dose of UVB on non-involved psoriatic skin and given oral retinoid (NIPS-R). The other was treated with a single dose of UVB non-involved psoriatic skin without retinoid (NIPS).

Twenty non-psoriatics were taken to assess the density of LC in non-psoriatic healthy skin (NPHS). Seven of NPHS were further treated with a single dose of UVB to determine the density of LC after UVB in normal healthy subjects.

Medication

An aromatic retinoid, etretinate (RO 10-9359, Tigarson®, Hoffmann La Roche, Grenzachwyhlen) at a dosage of 40 mg per day was orally administered for 24 days to NIPS-R.

UVB irradiation

A bank of 13 FS-40 tubes (Waldmann, model UV 8001k, West Germany) emitting photons over the range 290-320 nm, with a peak output at 310-315 nm, was the UVB source. The dose of 110 mJ/cm^2 corresponds to MED testing in 25 healthy subjects by our light source. NIPS-R, NIPS and NPHS were irradiated on non-diseased skin of the left buttock area with UVB in a single dose of MED.

Skin biopsy

Non-diseased skin was taken with 4 mm punch at both side (left: irradiated, right: non-irradiated) of buttock in NIPS-R, NIPS, and seven NPHS on days 1, 8, 15 and 24 after UVR. The skin biopsy of NPHS without UVR was also taken from either side of buttock.

Detection of T6$^+$ and HLA-DR$^+$ cells in epidermal sheets

Fat-trimmed tissue specimens were immersed in 1N sodium bromide solution for 60 min. at 37°C. The epidermis was subsequently teased off the dermis with fine forceps and washed in phosphate buffered saline (PBS) prior to immunoperoxidase staining.

Anti-T6 Mcab (OKT6), anti-HLA-DR Mcab (OKDR) and ortho universal immunoperoxidase kit (Ortho diagnostics, Raritan, New Jersey) were used as described in table 1.

Table 1. Staining procedure for identifying LC (Using a three step labelling method)

Fix the epidermal sheets in acetone for 30 min.
Normal serum (same species as linking reagent) in 0.01 M PBS buffer containing 0.01% sodium azide for 60 min.
Primary anitbody (Mcab, OKT6 and OKDR, 1:40 dilution using PBS with 0.1% sodium azide and 1% carrier protein) to 60 min.
Anti-mouse immunoglobulin serum for 60 min.
Peroxidase labeled mouse immunoglobulins in 0.01 M PBS buffer and 1% carrier protein for 60 min.
Substrate solution (2 ml of acetate buffer, 1 dorp of 3-amino-9-ethylcarbazole in N, N-dimethyl water) for 20 min.

Counting method

The cells showing brownish-red stained nucleus and dendrites were counted as LC. In the first step, photographs were taken under x100 light microscope. Three randomly chosen, non-overlapping fields in the photographs (one field at a magnification of x100 corresponds to an area of 0.06 mm^2) were counted and their numbers of LC x 50/9 were adjusted per square milimeter of epidermal area.

RESULTS

Density of T6⁺ LC vs. HLA-DR⁺ LC in NPHS and NIPS

T6⁺ LC and HLA-DR⁺ LC were distributed uniformly in all preparations of epidermal sheets (Fig. 2, 7, 12 & 17).

Table 2. T6⁺ LC and HLA-DR⁺ LC in NPHS and NIPS

	T6⁺ LC (No. of positive cells/mm^2)	HLA-DR⁺ LC	HLA-DR⁺/T6⁺ LC
NPHS (n=20)	539±16	446±35	83%
NIPS (n=16*)	474±40	378±53	80%
Statistical** significances of NPHS vs. NIPS	$p<0.01$	$p<0.05$	

NIPS: Non-involved psoriatic skin
NPHS: Non-psoriatic healthy skin
*8 NIPS-R before retinoid intake were included
**Wilcoxon rank sum test

Table 3. Time course of T6⁺ LC and HLA-DR⁺ LC density of NPHS (n=7) after UVB (110 mJ/mm^2)

Day*	T6⁺ LC (No. of positive cells/mm^2)	HLA-DR⁺LC
Control	528±30	435±35
1	175±72	164±121
8	252±96	198±75
15	327±64	252±48
24	429±28	343±37

* Days after a single dose of VUB

The densities of T6⁺ LC and HLA-DR⁺ LC were greater in NPHS than NIPS. The proportion of HLA-DR⁺ LC to T6⁺ LC in NPHS and NIPS were 83% and 80% (Table 2).

Effect of UVB on LC

Time course of UVB effect on T6⁺ and HLA-DR⁺ LC density was studied (Table 3). The density of T6⁺ LC as well as HLA-DR⁺ LC was reduced markedly within 1 day and recovered gradually (Fig. 3-6 & 13-16). As density of LC diminished, the dendritic process became shorter or disappeared and the central body became oval or round. Incomplete recovery the number of cells, approximately 80% of unirradiated site, with restoration of regular distribution and morphology of T6⁺ LC and HLA-DR⁺ LC was observed 24 days after UVR (Fig. 6 & 16).

Effect of UVB in NIPS-R

The density of T6⁺ LC and HLA-DR⁺ LC at days 0, 1, 8, 15 and 24 after UVR in NIPS-R was compared with the NIPS (Fig. 1). After showing a similar pattern of depletion of LC following UVR, sharper recovery of LC was seen in NIPS-R than NIPS. The LC of NIPS-R recovered to pre-UVR level in number, distribution and morphology 24 days after UVR (Fig. 7-11 & 17-21).

DISCUSSION

The densities of HLA-DR⁺ LC in epidermis reported by other investigators (4.87±2.03 per mm^2 of epidermal sections, Fernandez-Bussy et al., 1983; 15 per linear mm, Mackie & Turbitt, 1983; 19.3±5.2 per 0.1 mm^2, Claudy & Rouchouse, 1984) were lower than our result (446±35/mm^2) when their results were recounted per square milimeter. These results may be due to the difference in tissue preparation for staining. We used epidermal sheets for tissue preparation which is known as superior to frozen sections in that it covers the whole epidermis and also better expresses surface markers of LC (Berman et al., 1983a).

Harrist et al. (1983) observed that HLA-DR antigen was detectable in 18-63% of the T6 antigen bearing LC in normal human epidermis. Berman et al. (1983b) stated that approximately 50% of T6 antigen bearing LC did not show any detectable level of HLA-DR antigen. Mackie and Turbitt (1983) stated that two antibodies used to delineate LC may not label identical dendritic epidermal cell population either in normal and diseased skin. These

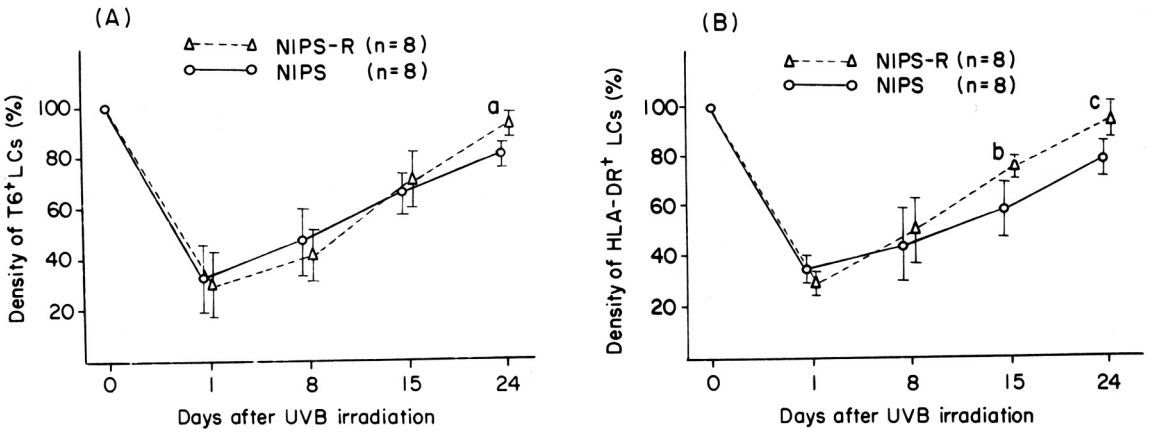

Fig. 1. Time courses of $T6^+$ LCs (A) and HLA-DR^+ LCs (B) in NIPS-R and NIPS after UVB.
a, b, c: significant difference (Wilcoxon rank sum test, $p<0.01$) from NIPS.

studies suggested that HLA-$DR^+/T6^+$ and HLA-$DR^-/T6^+$ subsets of LC exist in epidermis.

Recently, Liu et al. (1986), using parallel double-staining techniques on epedermal sheets as well as on frozen sections as a control, reported that 97-100% of $T6^+$ LC were HLA-DR^+. This discrepancy, they say, may be due to the difference of tissue preparation and labelling method, and the HLA-DR antigen which is more fragile than T6 antigen.

In our current study, HLA-DR^+ LC was approximately 80% of $T6^+$ LC. Even that showed the greatest number of HLA-DR^+ LC was still less than the smallest number of $T6^+$ LC observed among all epidermal sheets. Our result concur with Berman et al. (1985) in that the total number of HLA-DR^+ LC never exceed $T6^+$ LC.

The exposure of UVB or PUVA depleted LC density even with doses of irradiatioin producing no visible erythema (Kalimo et al., 1983) and a single dose of 60-80 mJ/cm^2 in the UVB spectrum produced a virtually complete elimination of LC markers (Aberer et al., 1981). Our current study indicated that, in both normal and psoriatic subjects, a single dose of 110 mJ/cm^2 of UVB equivalent to MED reduced number of LC membrane marker to approximately 30% of non-irradiated control skin. In our previous study (unpublished data), even by 300 mJ/cm^2 of UVB did not totally eliminated LC markers in normal Koreans.

Aromatic retinoid, particularly etretinate, have proved to be beneficial in skin disorders such as psoriasis characterized by disturbed epidermal proliferation and keratinization. The remedy of combined etretinate and UVB irradiation or PUVA leads frequently to the remission of psoriasis, and these treatment may reduce the treatment period as well as the amount of irradiation required for the remission (Fritisch et al., 1978; Orfanos et al., 1979). Moreover, retinoid had turned out to increase the number of LC, in contrast to UVR which deplete the cell markers (Fernandez-Bussy et al., 1983; Haftek et al., 1983). The data furnished by our current study demonstrated that the recovery of $T6^+$ and HLA-DR^+ LC in NIPS-R was significantly faster than NIPS. Therefore, retinoid may be responsible for prompt recovery or normalization of LC from the UV induced depletion.

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Explanation of figures

Fig. 2. Non-irradiated psoriatic control (non-retinoid group) stained with anti-T6 Mcab (TC). Distinct dendritic $T6^+$ LC were homogeneously distributed within the whole preparation. The density of LC was 517 per mm^2. x 100.

Fig. 3. TC 1 days after UVB (110 mJ/cm^2) in a single dose showed marked depletion of $T6^+$ LC and dendritic processes were shortened and the central body became oval or round. Number of cells were 51 per mm^2. x 100.

Fig. 4-5. TC 8 and 15 days after UVB irradiation respectively. The number of $T6^+$ cells were gradually increased. x 100.

Fig. 6. TC 24 days after UVB showed incomplete recovery in the number of cell, but with a restoration of regualar distribution. Number of cells were 433 per mm^2. x 100.

Fig. 7. Non-irradiated psoriatic patient (retinoid group) stained with anti-T6 Mcab (TR). The distribution and morphology of $T6^+$ LC was similar to that of TC.

Fig. 8. TR 1 day after UVB (110 mJ/cm^2) in a single dose showed marked depletion $T6^+$ LC. Number of cells were 54 per mm^2. x 100.

Fig. 9-10. TR 8 and 15 days after UVB irradiation respectively. The number of $T6^+$ cells were gradually increased. x 100.

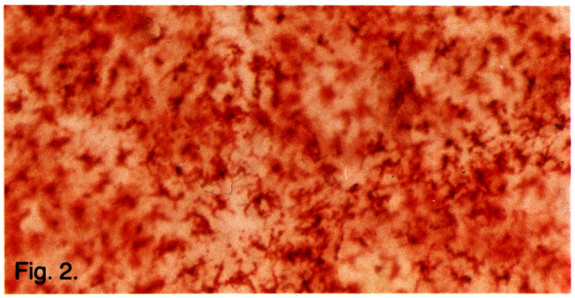
Fig. 11. TR 24 days after UVB. $T6^+$ LC recovered to the level of non-irradiated control in its distribution, population and morphology. x 100.

Fig. 12. Non-irradiated psoriatic control (non-retinoid group) stained with anti-HLA-DR Mcab (DC). HLA-DR^+ LC was evenly distributed and, in this particular epidermal sheet, the density of DR^+ cells corresponds to 87% of $T6^+$ LC of TC. x 100.

Fig. 13-16. DC 1, 8, 15, and 24 days after UVB (110 mJ/cm^2) irradiation respectively. The pattern of recovery from the depletion following the UVR was similar to TC. x 100.

Fig. 17. Non-irradiated psoriatic patient (retinoid group) stained with anti-HLA-DR Mcab (DR) showed distinct HLA-DR^+ LC distributed evenly. The density of HLA-DR^+ LC was 83% of TR. x 100.

Fig. 18-21. DR. 1, 8, 15 and 24 days after UVR respectively. The pattern of recovery from the depletion was similar to TR. x 100.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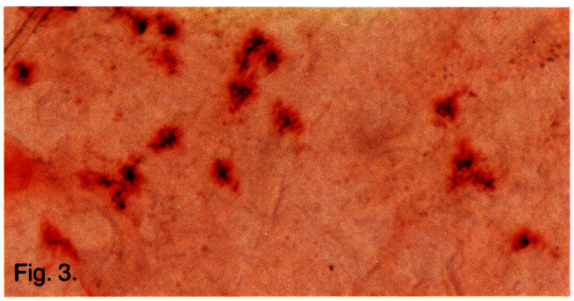
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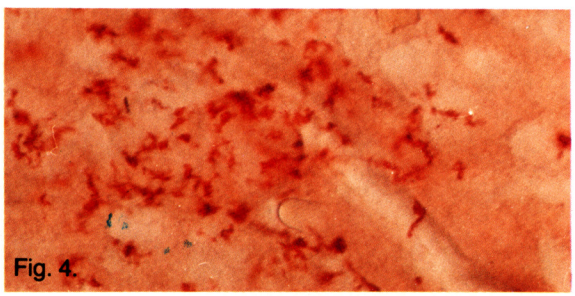
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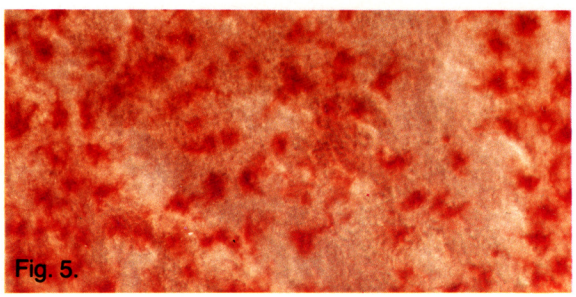
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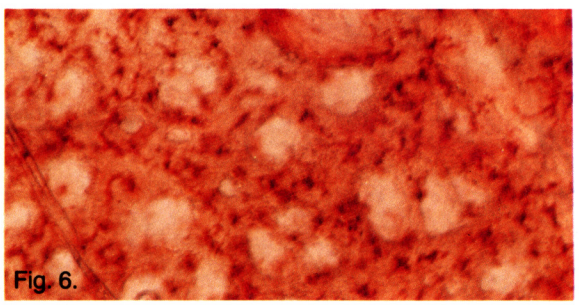
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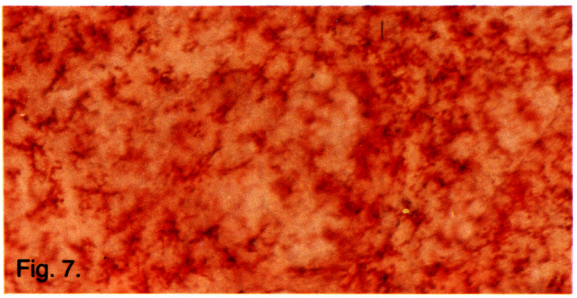
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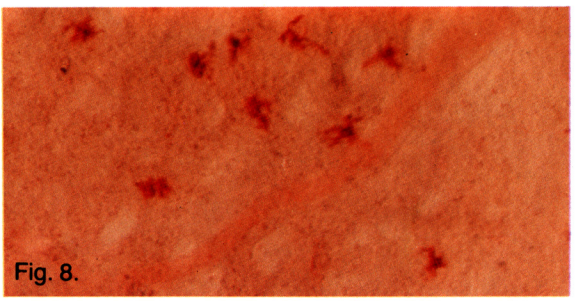
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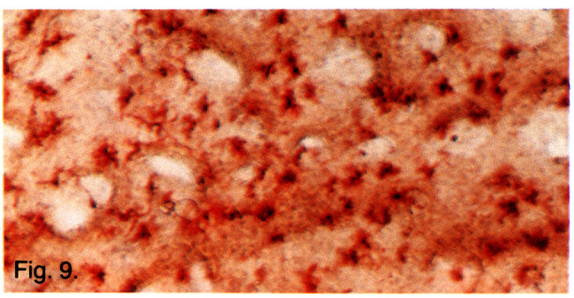
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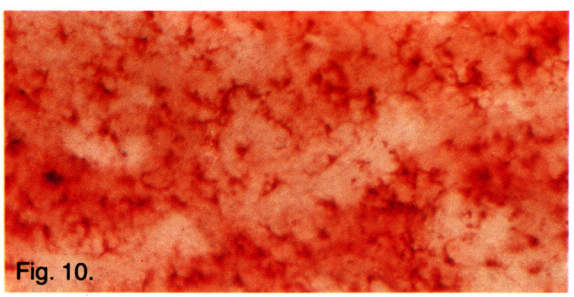
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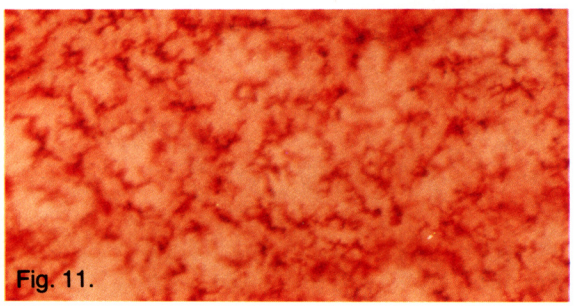
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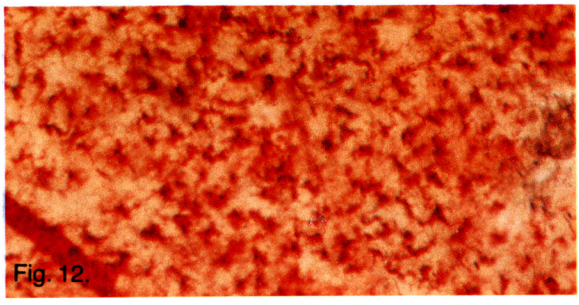
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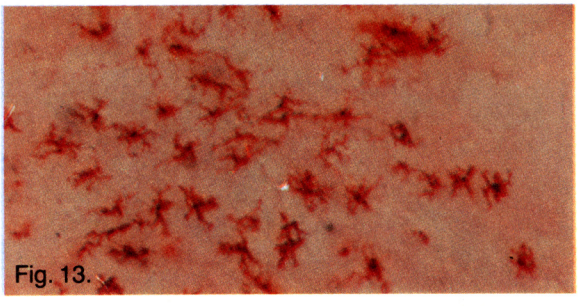
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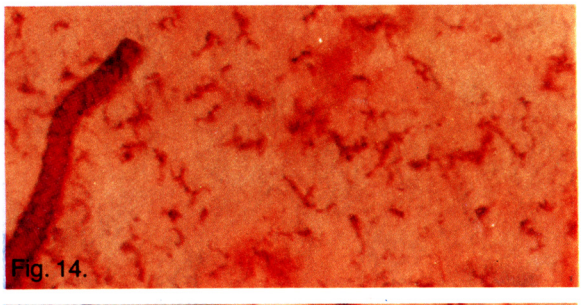
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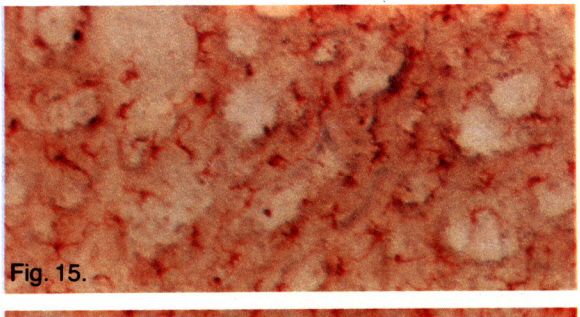
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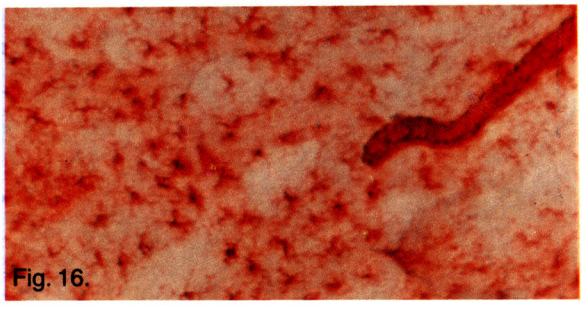
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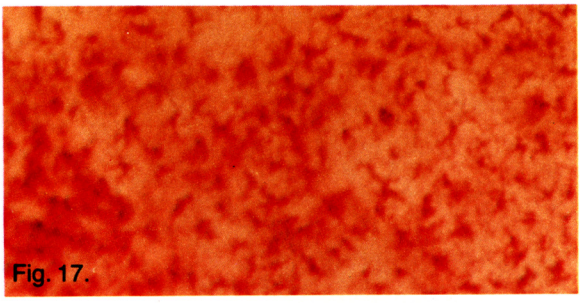
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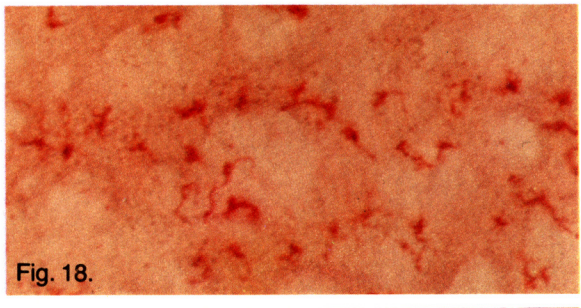
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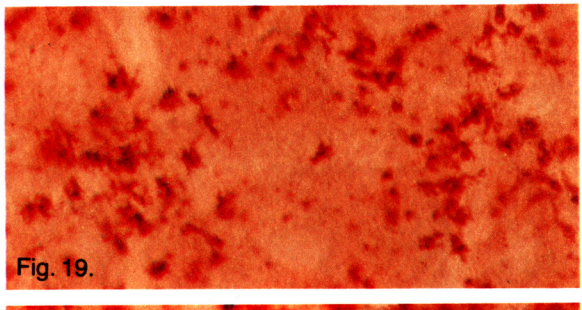
Fig. 19.

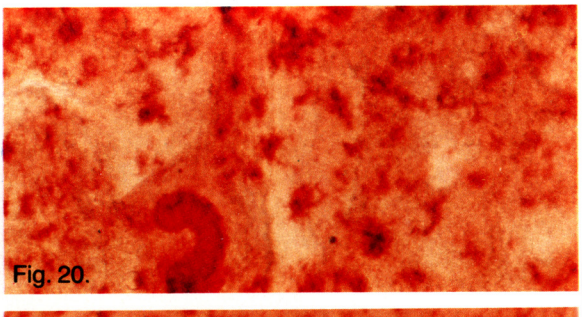
Fig. 20.

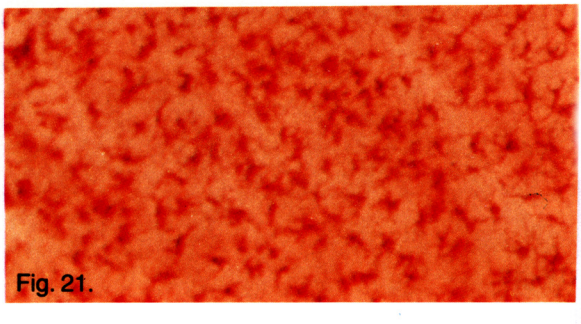
Fig. 21.