

## APPOSITION OF MONONUCLEAR CELLS TO LANGERHANS CELLS IN CONTACT ALLERGIC REACTIONS<sup>1</sup>

### *An Ultrastructural Study*

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**Abstract.** The ultrastructural features of Langerhans cells are described in positive contact allergic and contact irritant reactions and in clinically negative patch test sites. Skin biopsies of positive patch test reactions to nine different allergens in 21 allergic subjects were examined by electron microscopy. As controls, skin biopsies were studied 1) of clinically non-reactive patch test sites to five different allergens in 23 subjects, 2) of contact irritant reactions in 5 subjects, and 3) of lesions of 9 subjects with various dermatoses. Close apposition of mononuclear cells to Langerhans cells was seen almost exclusively at sites of contact allergic reactions. Some of these Langerhans cells showed prominent Golgi complexes, channels of rough endoplasmic reticulum, polyribosomes, lysosome-like bodies, and ruffled cell membranes with pseudopod-like projections. Still other Langerhans cells showed disruption of their cell membranes. The results of this study suggest that the Langerhans cell, for which no function is known, plays a role in contact allergic reactions. The nature of this role requires further study.

of clinically non-reactive patch tests, of contact irritant reactions, as well as biopsies from a few dermatoses.

### MATERIALS AND METHODS

#### *Human subjects*

Twenty-one subjects with *contact allergic reactions* to nine different substances were studied. Table I lists the substances applied, the number of subjects tested, the degree of reaction 48 hours after application of the allergen, and the time of biopsy. Most of these subjects were clinically free of their dermatitis at the time of patch testing, although they had had some clinical manifestations of their sensitivity within 1 month prior to the testing.

Five subjects with *contact irritant reactions* were studied. Table II lists the substances applied, the number of subjects tested with each substance, the degree of reaction, and time of biopsy.

Twenty-three subjects with *clinically non-reactive patch test sites* were studied. Table III lists the substances applied, the number of subjects tested with each substance, and the time of biopsy.

Prior to inclusion in this study, each subject had been patch tested and the site read at 48 and 72 hours. In non-reactive subjects the patch tests were repeated after the studies were concluded, to exclude the possibility that subjects had become sensitized to the allergen. Patch tests were graded 0 to 4+ according to accepted methods (15).

The lesions of 9 subjects with a *variety of dermatoses* were also studied. The clinical conditions and number of patients examined are listed in Table IV.

#### *Preparation of skin biopsy specimens for electron microscopy*

Most specimens were fixed in 4% phosphate-buffered glutaraldehyde for 2 hours at 4°C. Of these, tissue blocks were then washed in two changes of cold isotonic saline

Several investigators have suggested the possibility that Langerhans cells play a role in contact allergic reactions (1, 5, 7). In previous studies of allergic contact reactions by electron microscopy (13), I reported close apposition of mononuclear cells to Langerhans cells as early as 4 hours after topical application of mercury bichloride and signs of damage to some Langerhans cells by 48 hours. The objective of the present study was to ascertain whether apposition also occurs at sites of contact allergic reactions to other allergens. In addition I studied sites

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Table I. Contact allergic patch test reactions

Substances applied	No. of subjects tested	Degree of patch test reactivity	Time of biopsy (hours)
Ammoniated mercury 10% in pet.	1	3+	24
Formaldehyde 2% aq. sol.	1	2+	24
Gold chloride 1/2% aq. sol.	1	1+	26
	1	2+	24
Hexachlorophene 1% in pet.	1	3+	4
Mercaptobenzothiazole 1% in pet.	1	4+	48
Mercury bichloride 0.1% aq. sol.	1	1+	24
	1	1+	72
	1	2+	2
	1	2+	24
	1	3+	2
	2	3+	4
	1	3+	24
	2	4+	48
Nickel sulfate 5% aq. sol.	1	2+	48
Paraphenylenediamine 2% in pet.	1	1+	24
	1	2+	24
	1	4+	24
Pyrethrum as is	1	3+	48

for 5 minutes and post-fixed for 1 hour in cold 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.4. Specimens were washed in three changes of cold isotonic saline solution, dehydrated in graded alcohols, and embedded in epoxy resin (Maraglas). Silver to gold sections were cut from blocks treated in the above ways and placed on 200 mesh, Formvar-coated or uncoated stainless steel grids. Some sections were stained with uranyl acetate, lead citrate, or both. The sections were then examined by electron microscopy (Siemens Elmiskop 1A).

In addition, some specimens were processed in osmium zinc iodide as described previously (9). Sections approximately 2  $\mu$ m thick were cut for light microscopic study and counterstained with Azur II (6). Sections were also cut for study by electron microscopy.

## RESULTS

### Routine Studies by Electron Microscopy

#### Findings at sites of positive contact allergic reactions

In contact allergic reactions, mononuclear cells were seen juxtaposed to Langerhans cells 4 to 6 hours or longer after application of the allergen (14). Also, the following morphologic changes in Langerhans cells next to mononuclear cells were

frequently seen in contact allergic reactions: 1) Membrane-limited inclusions resembling vesicles; 2) Polyribosomes and prominent channels of rough endoplasmic reticulum containing faintly opaque material; 3) Prominent Golgi complexes; 4) Several multivesicular bodies and membrane-limited inclusions resembling lysosomes; 5) Glycogen accumulation; 6) Ruffled cell membranes with pseudopod-like projections; 7) Indistinct cell margins with disorganization of cytoplasmic components.

Table II. Contact irritant patch test reactions

Substances applied	No. of subjects tested	Degree of patch test reactivity	Time of biopsy (hours)
Mercury bichloride 1% aq. sol.	1	2-	24
Mercury bichloride 2% aq. sol.	1	3+	24
Soap	1	1+	48
	1	2-	24
Sodium lauryl sulphate 5% aq. sol.	1	1+	30

Table III. Clinically non-reactive patch test sites

Substance applied	No. of subjects tested	Time of biopsy (hours)
Ammoniated mercury 10% in pet.	2	48
Chromium chloride 2% aq. sol.	1	13
Formaldehyde 2% aq. sol.	1	48
Mercury bichloride 0.1% aq. sol.	2	4
	1	6
	3	12
	7	24
	3	48
	1	96
Paraphenylene diamine 2% in pet.	1	24
	1	48

Table IV. Dermatoses studied without patch testing

Clinical conditions studied	No. of patients studied
Lepromatous leprosy	1
Secondary syphilis	2
Erythema multiforme	2
Lichen planus	2
Contact dermatitis	2

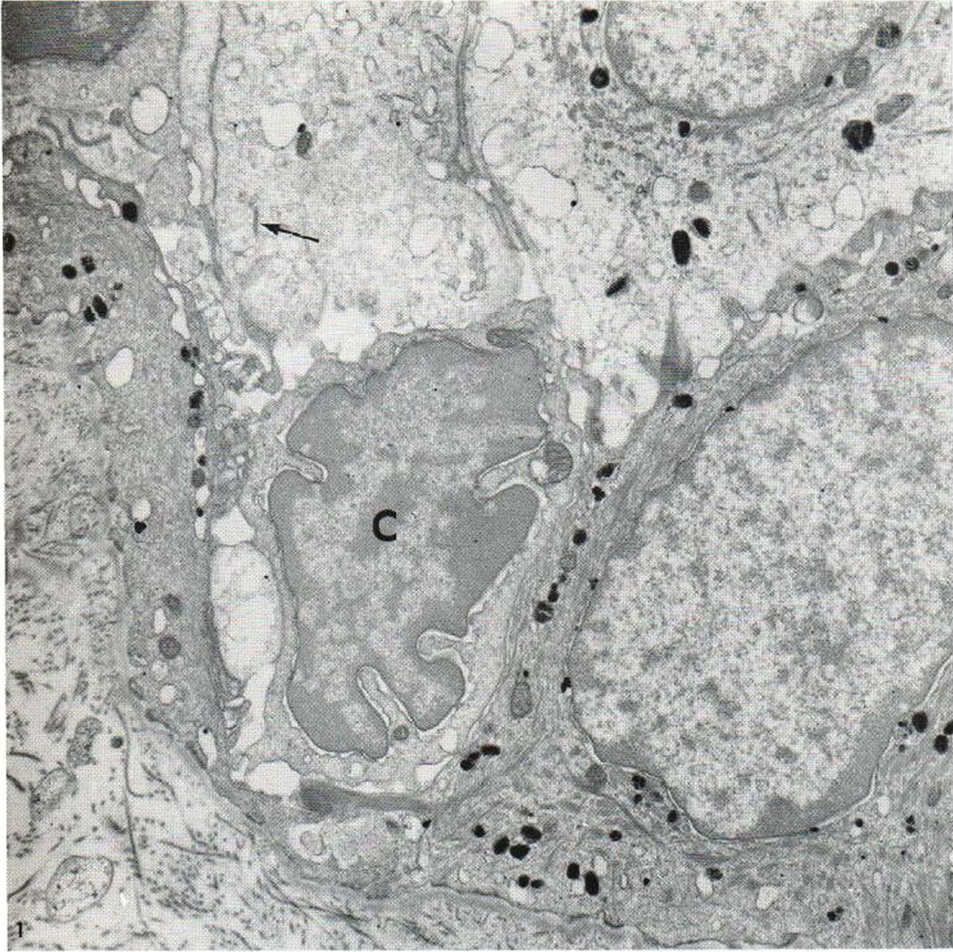


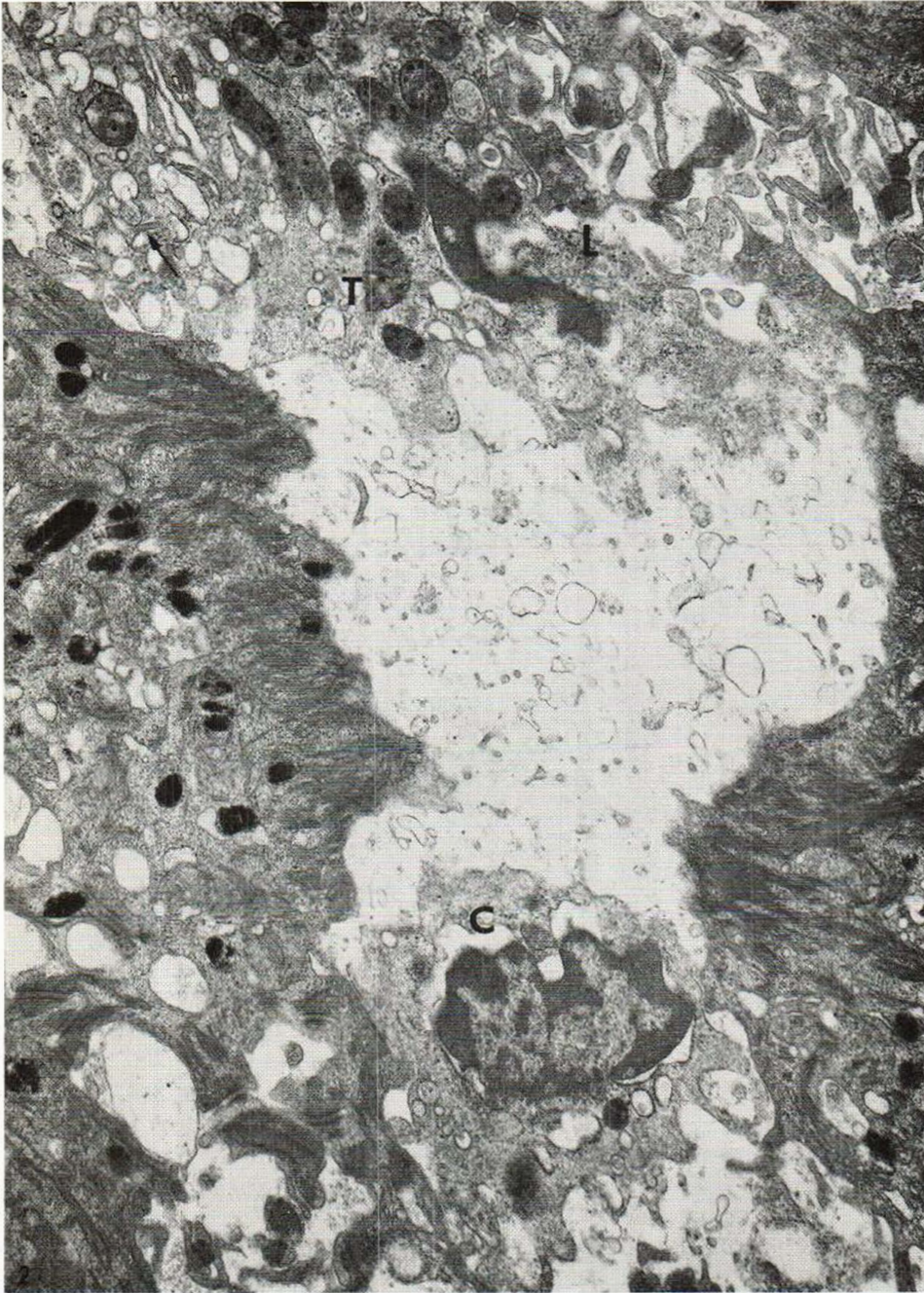
Fig. 1. Mononuclear cell (C) juxtaposed to dendrite of a Langerhans cell. Arrow indicates a Langerhans granule. At site of apposition, ribosomes and inclusions resembling vesicles are seen in cytoplasm of both cells. Because of

relatively large nucleus and scanty cytoplasm the mononuclear cell resembles a lymphocyte. Section stained with uranyl acetate and lead citrate,  $\times 11\ 500$ .

In some sites Langerhans cells were seen in the dermis, again in apposition to mononuclear cells. The accompanying figures illustrate some of these changes. Fig. 1 shows a mononuclear cell juxtaposed to the dendrite of a Langerhans cell. Because of the relatively large nucleus and scanty cytoplasm the mononuclear cell resembles a lymphocyte. In the cytoplasm of the cells at the site of apposition, ribosomes and inclusions resembling vesicles are present. Fig. 2 shows damage to a Langerhans cell that is in apposition to a mononuclear cell. Occasionally, as seen in Figs. 3, 4 and 5 more than one mononuclear cell may be juxtaposed to a Langerhans cell.

#### *Findings at sites of positive contact irritant reactions*

In none of five cases of contact irritant reaction was juxtaposition of mononuclear cells to Langerhans cells observed. As shown in Fig. 6, at the height of the contact irritant reaction the cell margins of the Langerhans cell are intact, but there is some glycogen accumulation. Although mononuclear cells are present they are not in juxtaposition to Langerhans cells. In one non-sensitive subject, in addition to testing with the irritant concentration of 2% mercury bichloride, the non-irritant concentration of 0.1% mercury bichloride was applied at another site. Only at



*Fig. 2.* Damaged Langerhans cell (L) at site of apposition to a mononuclear cell (C). The membrane profiles probably represent parts of degenerating Langerhans cell cytoplasm spilling over into this area. A Langerhans granule is indicated by the arrow. Mitochondria (T)

show electron-dense deposits. The mononuclear cell also shows indistinct cell margins, possibly the result of injury by hydrolytic enzymes released by the damaged Langerhans cell. Section stained with uranyl acetate and lead citrate,  $\times 17\,000$ .



*Fig. 3.* Two mononuclear cells (C) juxtaposed to a Langerhans cell (L) which, unlike that seen in Fig. 2, shows very few degenerative changes. The area demarcated by lines contains Langerhans granules and is seen at

higher magnification in inset. Section stained with uranyl acetate and lead citrate,  $\times 15\,500$ . *Inset:* Two Langerhans granules,  $\times 27\,000$ .

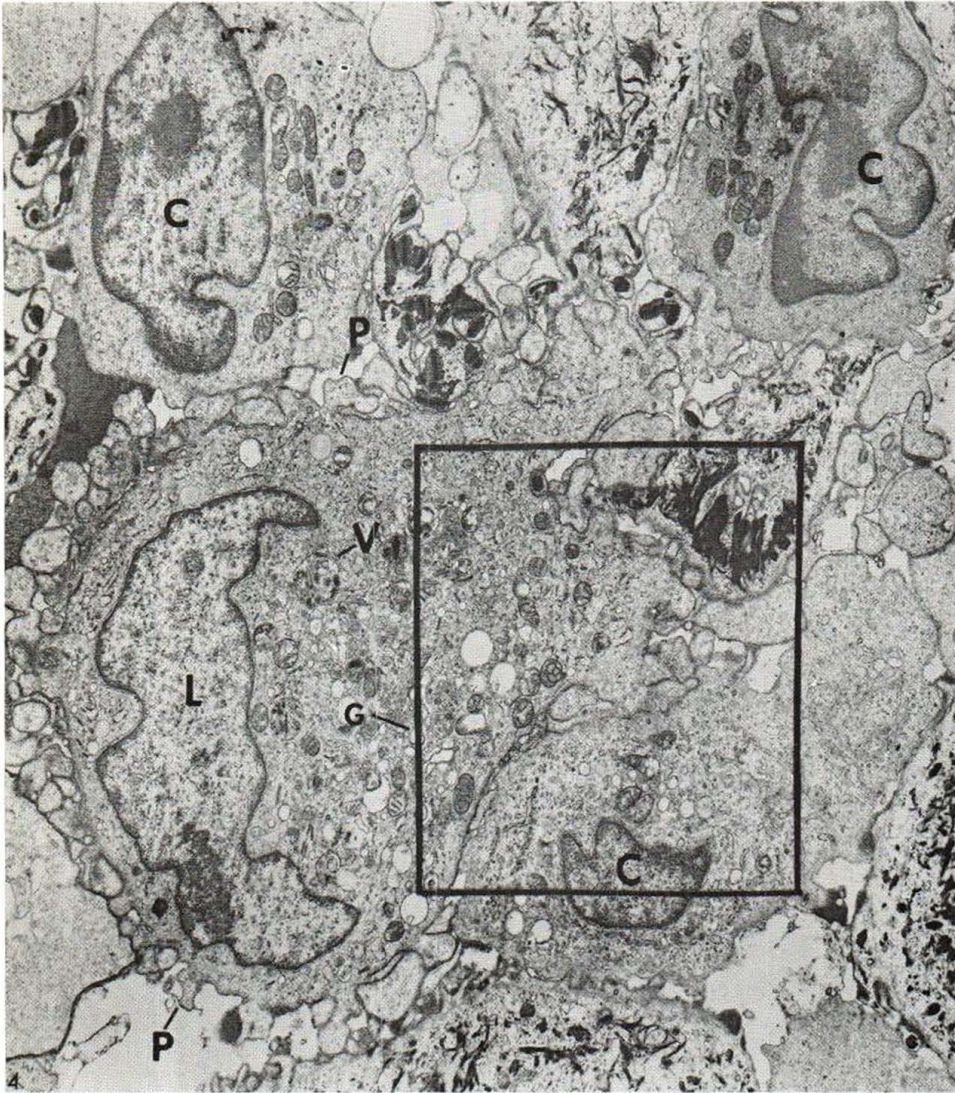


Fig. 4. Langerhans cell (L) surrounded by three mononuclear cells (C). Plasma membrane of the Langerhans cell is ruffled with many pseudopod-like projections (P). Lysosome-like bodies (V) and a prominent Golgi complex

(G) are found in the cytoplasm. The area demarcated by the lines is shown at higher magnification in Fig. 5. Stained with uranyl acetate and lead citrate,  $\times 7200$ .

this latter site of clinically negative reaction was apposition of mononuclear cells to Langerhans cells seen.

*Findings at sites of clinically non-reactive patch test reactions*

Apposition of mononuclear cells to Langerhans cells was not seen at non-reactive patch test sites with the exception of mercury bichloride. As shown in Table V this phenomenon occurred less

frequently at non-reactive patch test sites to mercury bichloride than at sites of contact allergic reactions. As reported previously, Langerhans cells at these sites show signs of injury, and occasionally keratinocytes are seen that contain Langerhans cell organelles, probably of phagocytic origin (12). In none of the six non-reactive patch test sites to allergens other than mercury bichloride was juxtaposition of mononuclear cells to Langerhans cells seen.

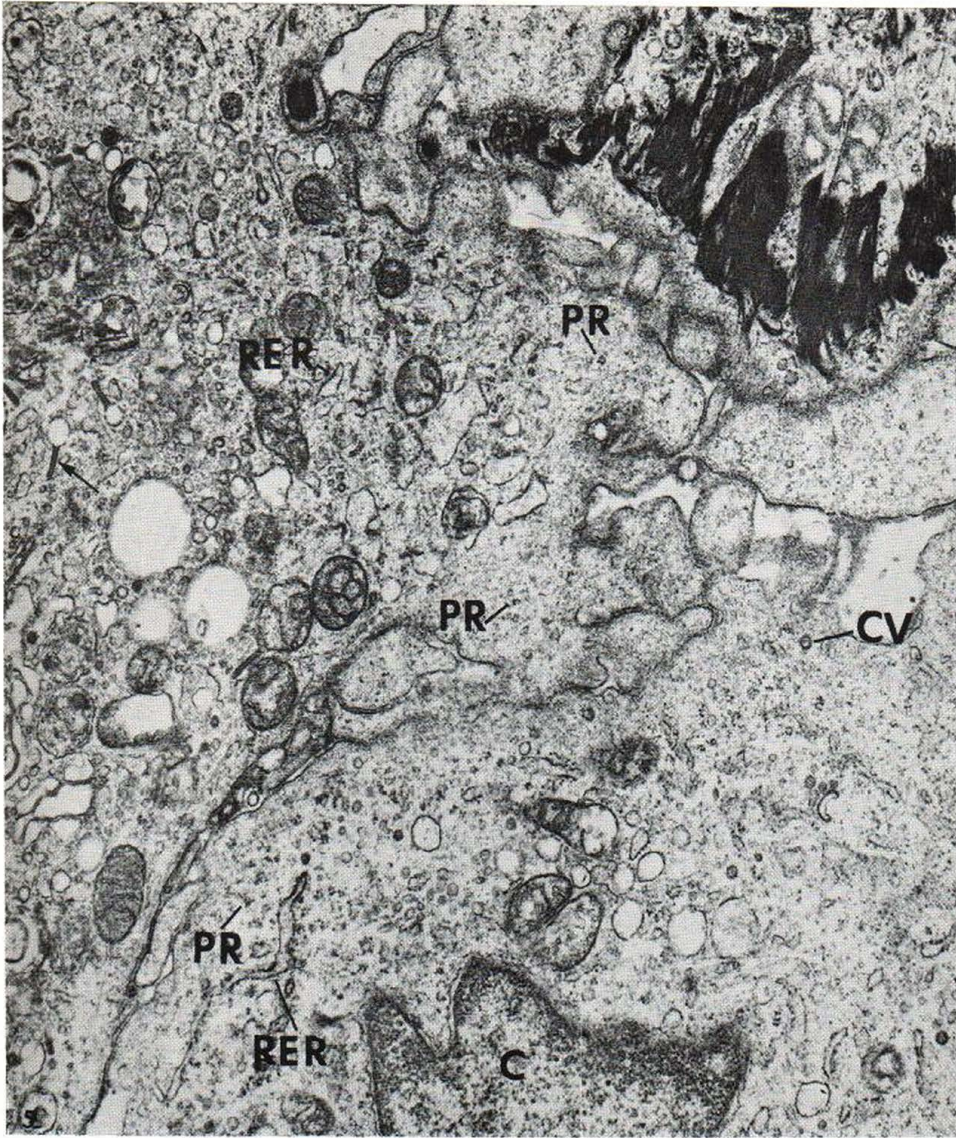


Fig. 5. Enlargement of part of Fig. 4. Arrow indicates a Langerhans granule. Polyribosomes (PR) and channels of rough endoplasmic reticulum (RER) are present in cytoplasm of Langerhans cell. In an area adjacent to

the Langerhans cell, the cytoplasm of a mononuclear cell (C) contains polyribosomes (PR), channels of rough endoplasmic reticulum (RER) and coated vesicles (CV).  $\times 18\ 300$ .

*Findings in lesions of other dermatoses*

In 2 subjects with chronic contact dermatitis, apposition of mononuclear cells to Langerhans cells was seen. Fig. 7 shows changes in a Langerhans cell so related to a mononuclear cell. Several membrane-limited inclusions containing amorphous material are seen. The cytoplasm is dark and contains ribosomes and prominent chan-

nels of rough endoplasmic reticulum containing moderately opaque material. The Golgi complex is prominent. Except for the presence of lysosome-like bodies these cells resemble those described by Ebner & Niebauer (4). Of the other dermatoses studied only in one of two biopsies of subjects with syphilis was there apposition of mononuclear cells to Langerhans cells.

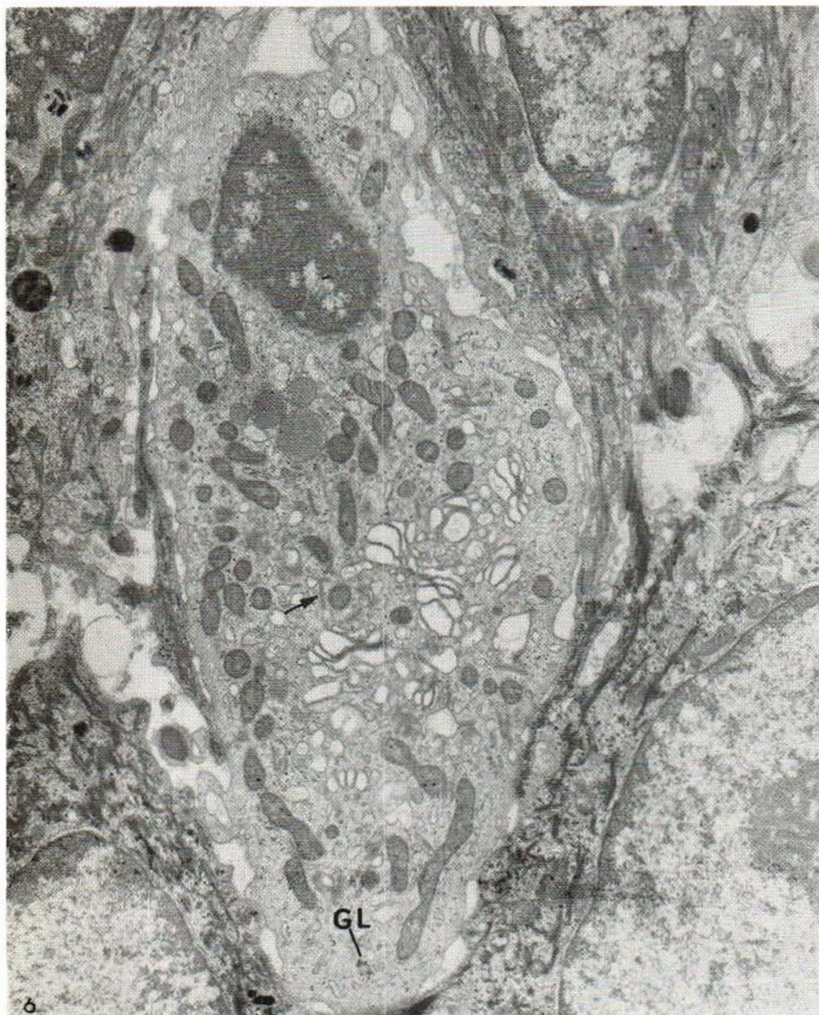


Fig. 6. Intact Langerhans cell except for some glycogen accumulation (GL). At site of contact irritant reaction to mercuric chloride a Langerhans granule is seen (arrow). Section stained with uranyl acetate and lead citrate,  $\times 10\ 900$ .

#### *Results of Osmium Zinc Iodide Staining*

At clinically non-reactive patch test sites, Langerhans cells were stained as described by Niebauer et al. (9). Langerhans cell counts by light microscopy of thick sections correlated well with electron microscopic findings. At sites of contact allergic and contact irritant patch test sites and in lesions of other dermatoses, staining of Langerhans cells was variable; Langerhans cell counts of thick sections by light microscopy correlated poorly with electron microscopic findings. Some Langerhans cells took almost no stain, whereas some keratinocytes and melanocytes were heavily stained. Attempts at quantitation of the apposition of mononuclear cells to Langerhans cells by light microscopy using the osmium zinc iodide stain

were unsuccessful. At sites of positive contact allergic reactions, some Langerhans cells showed osmium zinc iodide staining on parts of the cell membrane. When Langerhans cells were found in juxtaposition to mononuclear cells some staining was seen on the cell membrane of the Langerhans cells at the sites of apposition.

#### *Quantitative Studies by Electron Microscopy*

Six blocks, approximately  $1\text{ mm}^2$  in size, of each specimen were studied, and a total of at least ten epidermal Langerhans cells per specimen were counted. The results of these studies are listed in Table V, and show that in positive contact allergic patch test sites, after 4 hours, apposition of mononuclear cells was seen in 46





**Fig. 7.** Apposition of mononuclear cell (C) to dendrite of a Langerhans cell in a lesion of chronic contact dermatitis. The Langerhans cell contains membrane-limited inclusions resembling lysosomes (V), numerous ribosomes (R), and channels of rough endoplasmic reticulum (ER) filled with moderately opaque material.

The Golgi region (G) is prominent. The area containing Langerhans granules is demarcated by lines and is shown at higher magnification in the inset. Section stained with uranyl acetate and lead citrate,  $\times 12\ 000$ . *Inset:* Langerhans granules.  $\times 25\ 000$ .

out of 210 Langerhans cells counted. In positive contact-irritant patch test sites of 100 Langerhans cells counted, none showed apposition to mononuclear cells. In clinically negative patch test to allergens, other than mercuric chloride, none of 60 Langerhans cells counted showed apposition to mononuclear cells. Seventeen Langerhans cells out of 170 counted showed apposition to mono-

nuclear cells at sites of clinically non-reactive patch test sites to mercuric chloride. With the exception of one of two subjects with secondary syphilis, none of the 70 Langerhans cells counted in lesions of other dermatoses, were apposed to mononuclear cells. In secondary syphilis, one of 30 Langerhans cells counted showed apposition to a mononuclear cell.

Table V. Results of quantitative studies by electron microscopy

Conditions studied	Total no. of Langerhans cells counted	No. of Langerhans cells showing apposition to mononuclear cells
Positive contact allergic patch test sites	210	46
Positive contact irritant patch test sites	100	0
Clinically negative patch test sites (not including reactions to HgCl <sub>2</sub> )	60	0
Clinically negative patch test sites to HgCl <sub>2</sub>	170	17
Chronic contact dermatitis	20	4
Lepromatous leprosy	10	0
Secondary syphilis	30	1
Erythema multiforme	40	0
Lichen planus	20	0

## DISCUSSION

In previous studies by electron microscopy of allergic contact dermatitis, apposition of various cell types has been described. Medenica & Rostenberg (8) noted contact of monocytes and lymphocytes. Wolff & Braun-Falco (16) reported intimate contact of macrophages to surfaces of lymphocytes. In the present report apposition of mononuclear cells and Langerhans cells is described. Many of the mononuclear cells had a relatively large nucleus in relation to the amount of cytoplasm. They resembled lymphocytes, but on morphologic grounds, they could not be positively identified. Therefore, the general term mononuclear cell is used to describe them. With the exception of non-irritant concentrations of mercury bichloride, it was only at sites of contact allergic sites but not at clinically non-reactive sites or sites of contact irritant patch test reactions that apposition of mononuclear cells to Langerhans cells was seen. The findings at clinically non-reactive patch test sites to mercury bichloride may be related to the finding that mercury bichloride in certain concentrations, like phytohemagglutinin, can nonspecifically cause transformation of lymphocytes *in vitro* (11). This unusual property may account for our findings *in vivo*.

Of particular interest are the different results

obtained at sites of irritant and non-irritant applications of mercury bichloride in the same subject. There was no apposition of mononuclear cells to Langerhans cells at the site of application of a contact-irritant concentration of mercury bichloride, while juxtaposition of mononuclear cells to Langerhans cells was noted at the site of application of mercury bichloride diluted to a clinically non-irritating concentration. As a further control some dermatoses were chosen which had at least as many infiltrating cells as have contact allergic reactions. Our attempts with light microscopy using osmium zinc iodide staining to quantitate the frequency of apposition of mononuclear cells to Langerhans cells at sites of inflammation were unsuccessful. Therefore, attempts at quantitation were made by electron microscopy. Only one Langerhans cell of 30 counted in lesions of secondary syphilis was juxtaposed to a mononuclear cell. The absence of this apposition in other sites where large numbers of mononuclear cells were present helped rule out the likelihood that the apposition seen in contact allergic reactions was due to chance.

Additional reasons supporting the concept that the association of mononuclear cells and Langerhans cells in allergic contact reactions is significant are the following. First, this association has so far been seen in all biopsy specimens of contact allergic reactions taken after 4 hours. Apposition occurred even before the time when a clinical reaction was seen at the patch test site and when, microscopically, only moderate numbers of mononuclear cells were present. Second, some of the Langerhans cells themselves showed morphologic changes at the site of apposition. These consisted of prominent channels of endoplasmic reticulum, which is evidence of cell secretion, or damage evidenced by indistinct cell borders and disorganization of cytoplasmic components. Our quantitative data are preliminary and studies are in progress to refine the methods for quantitating the association of these cells. Whether or not the number of mononuclear cells juxtaposed to Langerhans cells can be correlated with the severity of the contact allergic reaction is being investigated.

In this report the apposition of Langerhans cell and mononuclear cells is emphasized. Apposition of other cell types probably belonging to the lymphoid and macrophage class was also seen.

However, I could not conclusively identify the cell types in tissue sections. Identification by functional and histochemical studies would be necessary to determine their identity.

The results of this study strongly suggest a functional relationship between mononuclear cells and Langerhans cells in contact allergic reactions. Several possible explanations present themselves. The first is uptake and processing of allergenic material by the Langerhans cells with transfer of an immunologically active fragment to mononuclear cells, presumably lymphocytes. In this way Langerhans cells would act like macrophages (5). The second is related to the findings of Wolff (17) and Sagebiel (10) that Langerhans cells may take up materials such as thorotrast and ferritin, but that they do so less efficiently than do keratinocytes. If similar events occur after application of other substances, more immunologically active material may be found at the plasma membrane of Langerhans cells than at the surface of keratinocytes. Mononuclear cells might then concentrate in these areas. The third, is related to the theory that Langerhans cells may migrate (5). "Migration inhibitory factors" released by stimulated lymphocytes (3) could inhibit this migration, thus resulting in apposition of mononuclear cells and Langerhans cells. The fourth, as suggested by Kuwahara, is that the Langerhans cell may be involved in early antibody production (7). Mononuclear cells may then be attracted to sites of Langerhans cells by antigen-antibody complexes.

The possibility that the Langerhans cell is secreting some substance in contact allergic reactions is suggested by the findings to date with osmium zinc iodide staining. Niebauer et al. (9) found that in normal epidermis the cell membrane of the Langerhans cell did not stain with osmium zinc iodide, but organelles within the cell did stain. Osmium zinc iodide staining of discrete areas on the cell membrane of Langerhans cells in contact allergic reactions may indicate that this material originated inside the cell and was then transported to the surface.

One apparent consequence of this juxtaposition is that some Langerhans cells show signs of damage. The hydrolytic enzymes contained in these cells (2) may be released as a result of cell injury and then produce further inflammatory changes in the epidermis.

The results of this study suggests that the Langerhans cell, for which no function is agreed upon, serves a function in the contact allergic reaction. The nature of this function requires further study.

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*Addendum*

Since submitting this manuscript, the following pertinent references have come to my attention:

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