

# The Histological and Immunohistochemical Pattern of Positive Patch Test Reactions to Gold Sodium Thiosulfate

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Gold sodium thiosulfate, inserted in the patch test standard series, gives a surprisingly high yield of positive reactions, about 10% of tested eczema patients, but with a low degree of clinical relevance. A histopathological study on patch test reactions was carried out in patients selected because of a combined contact allergy to gold sodium thiosulfate and nickel. Biopsies were taken from macroscopically similar reactions in dilution series of each allergen. The histological picture was clearly eczematous, without irritative features. In a blind comparison, test reactions induced by the two allergens could not be differentiated from each other. Long-lasting patch test reactions to gold sodium thiosulfate were characterized by an intense lymphocytic dermal infiltrate without epidermal involvement. Immunohistochemically, CD4+ and CD8+ T lymphocytes could be detected already 4-8 h post-challenge, and "naive" as well as "memory"-type T cells were demonstrated. Apparently, our findings reflect a true contact allergy to gold sodium thiosulfate. **Key words:** nickel sulfate; contact allergy.

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Gold in contact with human tissues has generally been regarded as an inert material. However, different gold preparations, used in e.g. rheumatoid arthritis, show a high frequency of cutaneous side-effects. Sporadic cases of allergic contact dermatitis and stomatitis due to metallic gold have also been published.

Recently, we introduced gold sodium thiosulfate (GTS) 0.5% pet. in our standard patch test series and immediately found a high frequency of positive tests to this monovalent gold salt (1). As a matter of fact, with about 9% of tested patients being positive, GTS is now our second most frequent allergen after nickel. The allergic nature of the positive tests to GTS was strongly supported by results from patch testing with a dilution series and with a chemically related compound, potassium dicyanoaurate, as well as from intracutaneous testing with GTS.

Clinically, the positive patch test reactions to GTS look like a classic expression of contact allergy. They may, however, be delayed with a maximal intensity at 6-8 days after application, sometimes even negative at 3 days. The test reactions may also be quite long-lasting, still active after 1-2 months. This phenomenon of persistent inflammation has previously been described for different gold salts including GTS (2-4).

A few patients with contact allergy to GTS have a history of sensitivity to metallic gold such as jewellery against skin or dental gold against mucous membrane. On the whole, however, the clinical relevance of positive GTS reactions was found to be low in a non-biased questionnaire study (5).

For these reasons we found it justified to undertake a compar-

ative study on the histological pattern of patch test reactions to GTS and nickel sulfate in selected patients with such a combined contact allergy. With immunohistochemical technique we also wanted to characterize the phenotype of the dermal inflammatory infiltrate as a way of further examining the possible allergic nature of the GTS reactions. We also studied some late epicutaneous reactions.

## MATERIAL AND METHODS

### Group 1

Ten patients tested with the standard patch test series and found positive to nickel sulfate (5.0% pet.) and to GTS (0.5% pet.) were asked to participate in a comparative study. (In our total material, there was no statistical correlation of positive tests between these two allergens (5)). There were one male and nine females aged 25-65 years. They were patch-tested with each allergen in a dilution series in water using a factor of  $\sqrt{10}$  for every step. Thus, the nickel sulfate series was 16.0% w/v, 5.0%, 1.6%, 0.5%, and 0.16%; the GTS series was 5.0% w/v, 1.6%, 0.5%, 0.16%, and 0.05%. Both dilution series started with higher concentrations than usually tested to get more positive reactions and hence increase the possibility to get equivalent test reactions from nickel and gold. One patient with a doubtful reaction to GTS in pet. at 0.5% but with a positive intracutaneous test when routinely tested was tested with a GTS dilution series starting at 16.0% w/v. These aqueous solutions with concentrations exceeding the routinely tested concentrations for nickel and gold have been tested in a great number of patients without yielding irritant reactions. The test technique was the use of Finn chambers<sup>®</sup> attached to Scanpor tape<sup>®</sup>, 15  $\mu$ l of the test solution being applied to each patch unit.

The patches were removed after 48 h and the tests were read after a further 24 h. From each patient 3-mm punch biopsies were taken from positive patch test reactions, one from the nickel and one from the GTS series, with the prerequisite that they should be as macroscopically similar as possible, irrespective of strong, weak or, as in patients No. 7 and 9, even negative. The most concentrated test solution giving a negative reaction was chosen in the latter cases. The dilution step and strength of reaction used for biopsy in each patient are given in Table I. All these biopsies were formalin-fixed and coded for a blind microscopic reading (HE-staining).

### Group 2

Seven patients with a previous positive patch test to GTS (0.5% pet.) were tested once again with the same technique, thereby eliciting ++ reactions. From each patient, two 3-mm punch biopsies were taken from the test site 72 h after application. One of the biopsies was fixed in 10% formalin for routine histological analysis. The other biopsy was embedded in Tissue-Tec OCT compound (Miles Inc. USA) and snap frozen in pre-cooled isopentane at -80°C. Six- $\mu$ m frozen sections were air-dried, fixed for 10 min in acetone and washed in PBS for 20 min. Endogenous peroxidase was reduced by 0.3% H<sub>2</sub>O<sub>2</sub> in PBS for 30 min followed by 1% normal rabbit serum (DAKO) in PBS for 30 min, to block non-specific background staining. The following monoclonal antibodies were applied for 60 min at room temperature at optimal dilutions: PD7 (anti CD45RB, "naive" lymphocytes, DAKO), Leu 18 (anti CD 45RA, "naive" lymphocytes, Becton-Dickinson), UCHL1 (anti CD 45RO, "memory" lymphocytes, DAKO), OKT4, 6 and 8 (CD4, CD1a and CD8, Ortho). Control sections were incubated without the primary

Table I. Individual test concentrations (% aq.) and results of reactions chosen for biopsy in 10 patients patch-tested with gold sodium thiosulfate (GTS) and nickel sulfate (- negative, + erythema and infiltration, ++ erythema, infiltration and papules, +++ erythema, infiltration, papules and vesicles)

Patient	Age/sex	GTS Conc.	Result	Nickel Conc.	Result	Note
1	27/F	0.16	+	0.16	+	
2	42/F	16	+	5	+	
3	36/F	1.6	+++	5	+++	
4	25/F	1.6	++	1.6	++	
5	46/F	0.05	++	0.16	++	Weak reactions
6	27/M	0.5	++	1.6	++	
7	57/F	5	-	0.5	-	GTS: pos. to 0.5% pet.; Ni: pos. to higher conc.
8	65/F	0.5	++	0.5	++	
9	61/F	0.16	-	16	-	GTS: pos. to higher conc.; Ni: pos. to 5% pet.
10	52/F	5	+	5	+	

antibodies. After a short rinse in PBS, sections were incubated with peroxidase-conjugated rabbit-anti-mouse IgG (DAKO) in PBS containing 5% normal rat serum. We used DAB for 30 min as chromogen, and the sections were counterstained with Mayer's htx and mounted.

### Group 3

In order to characterize the inflammatory infiltrate of very early test reactions to GTS, biopsies were taken for immunohistochemical analysis. Three patients were selected who, in addition to a positive patch test to GTS, had a contact allergy to other allergens, viz. patient A: formaldehyde, B: lanolin and Kathon CG, and C: nickel. In patients A and

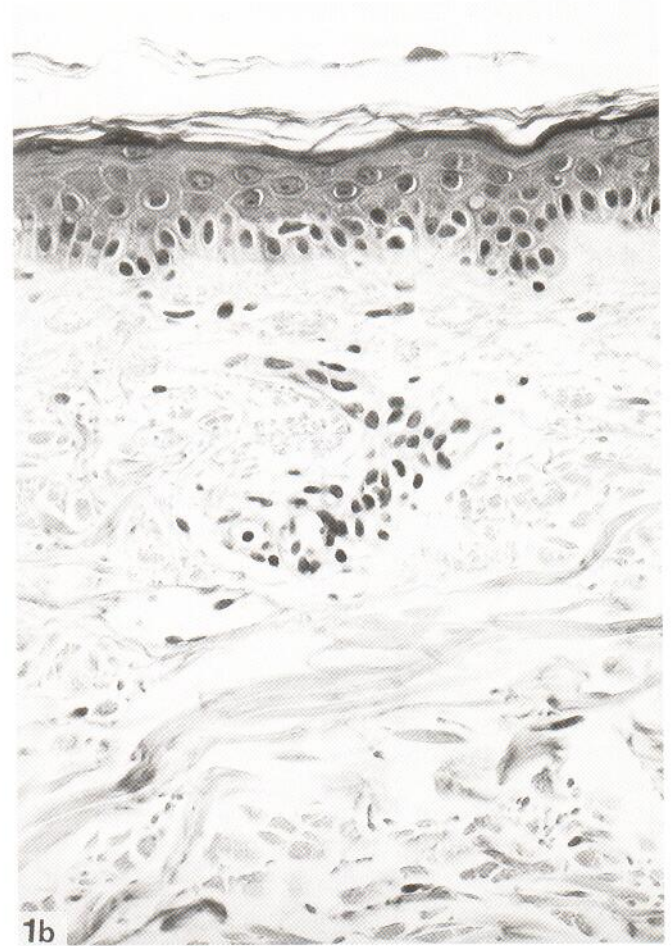


Fig. 1. (a) Clinically negative (grade 0) GTS patch test site (group 1), showing only a few lymphocytic cells around vessels in the papillary and reticular dermis (arrows). (b) At higher magnification, the lymphocytic cells are clearly seen to occur at vessels and only isolated cells are seen basally in the epidermis. The corresponding nickel test site (clinical grade 0) showed a similar type of reaction. HE-staining. (Fig. 1a  $\times 100$ , 1b  $\times 400$ ).

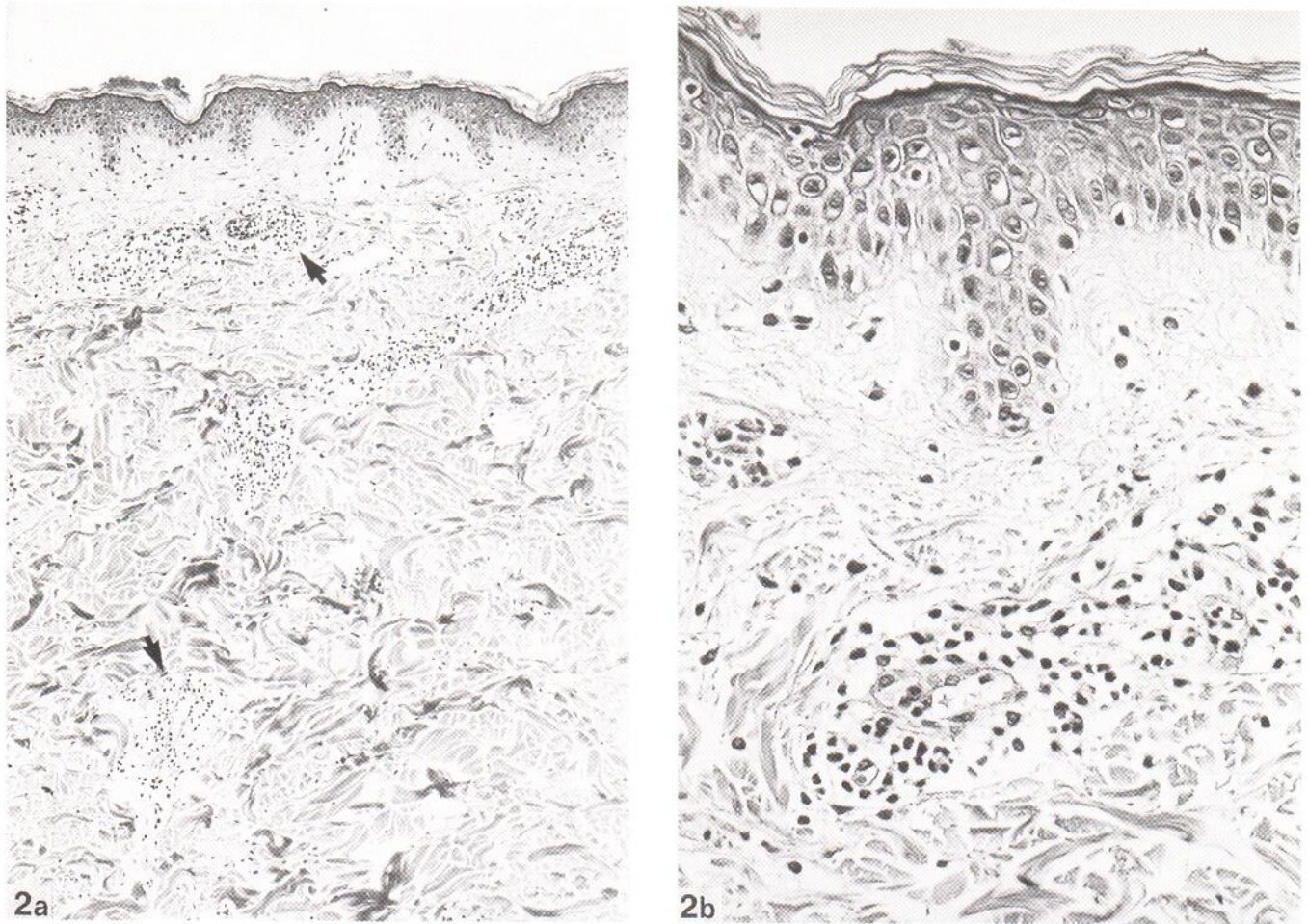


Fig 2. (a) Clinically positive (grade +) GTS patch test site (group 1). Perivascular lymphocytic infiltrates are clearly seen in the papillary and reticular dermis (arrows). In (b), higher magnification of Fig. 2a shows oedema of the papillary dermis, including a small number of lymphocytes in the epidermis accompanied by a slight degree of hydropic and spongiotic change. The corresponding nickel test site (grade +) showed a similar type of reaction. HE-staining. (Fig. 2a  $\times 100$ , 2b  $\times 400$ ).

B biopsies were taken from test reaction to GTS and other allergens 4 h after test application, in patients C after 8 h, i.e. well before any macroscopic reaction had appeared. A biopsy was also taken from a test-negative control subject 4 h after application of a GTS patch test. These biopsies were snap frozen and treated similarly to the freeze-fixed biopsies of group 2.

#### Group 4

Biopsies were also taken from 3 patients with long-standing positive patch test reactions to GTS. Since we had no systemic follow-up for such reactions, biopsies were taken when patients called to tell us about prevailing tests or when such reactions were noticed at a regular revisit. Thus, the biopsies were taken 2, 5 and 10 weeks respectively, after application of the original GTS test. These biopsies were routinely fixed by formalin.

## RESULTS

### Group 1

Two main types of tissue reactions in positive patch tests to GTS could be identified. In 2 of the patients (four biopsies), clinically negative reactions (grade 0) were accompanied by very discrete tissue changes. A few lymphocytic cells could be seen in the papillary and reticular dermis, particularly around superficial vessels. No differences were observed between the sites tested

with gold and nickel (Fig. 1). The remaining 8 patients (16 biopsies) showed +, ++ or +++ reactions. In these patients, a slightly variable degree of lymphocytic infiltration was found, clearly more intense than in the two previous cases. A small number of lymphocytic cells appeared in the basal epidermis and the adjacent dermis, often accompanied by some oedema. Apart from a slight thickening and a very discrete spongiosis, no other change was seen in the epidermis (Fig. 2). Further, perivascular lymphocytic infiltrates were found in the papillary and upper reticular dermis (Fig. 2), without involvement of deeply situated vessels. Major differences between the two biopsies of each individual pair (gold and nickel) of these 8 patients were difficult to determine. However, six of the biopsies, all with clinical grade 2–3 reactions (Fig. 3), showed a somewhat more intense lymphocytic infiltration than the other ten biopsies (clinical grade 1–2 reactions). Four of the six more intensely reactive biopsies had been taken from test reactions to gold, the other two from reactions to nickel.

### Group 2

All the seven biopsies showed the following features, with only a slight degree of variation. A few lymphocyte-like cells ap-

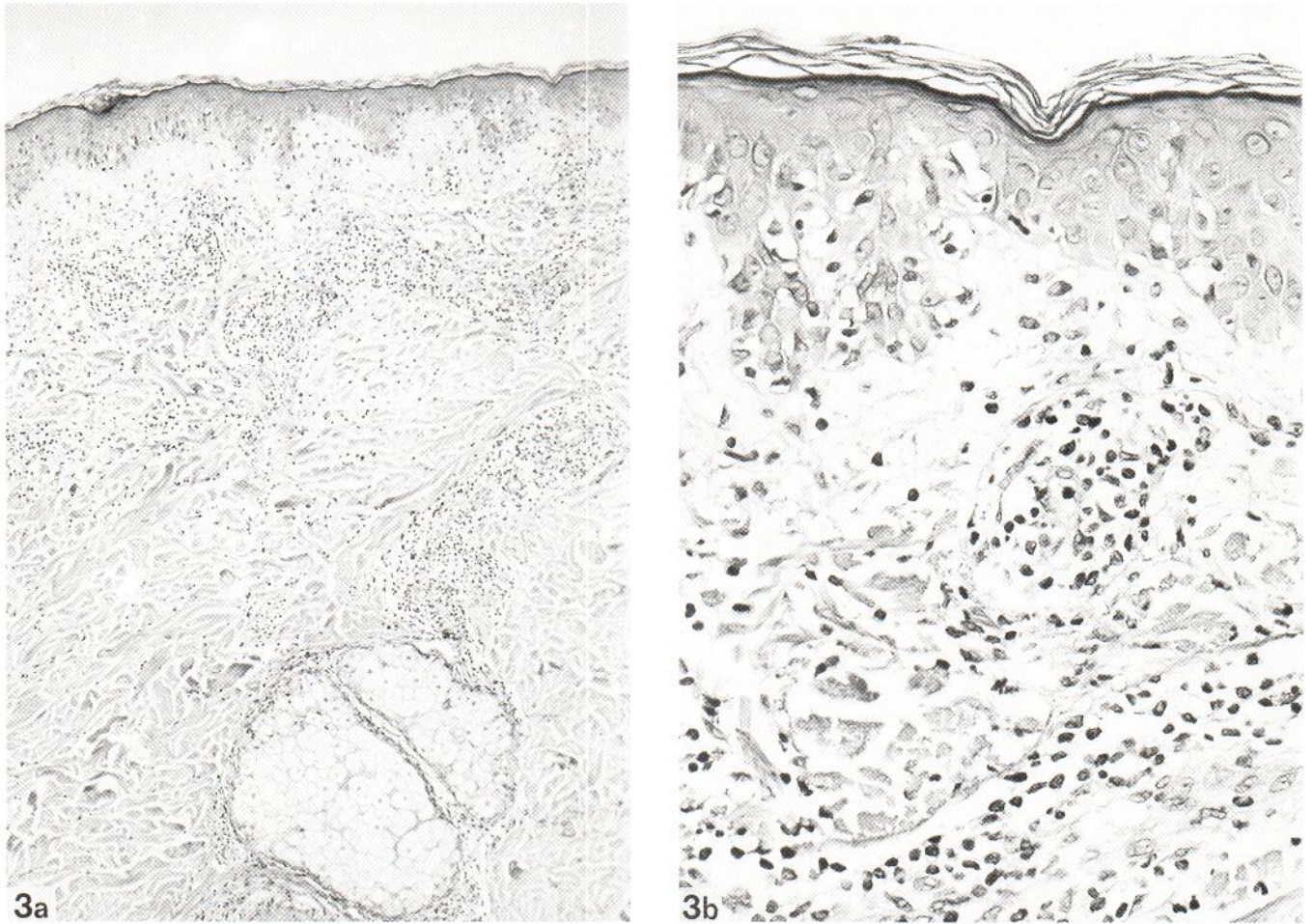


Fig. 3. (a) Clinically positive (grade +++) GTS patch test site (group 1), showing dense perivascular lymphocytic infiltrates in papillary and reticular dermis, also involving sebaceous glands. Higher magnification (b) shows oedema of papillary dermis, with lymphocytes also within the epidermis accompanied by hydropic and some spongiotic change. A similar type of reaction was seen in the corresponding nickel test site (grade +++) HE-staining. (a  $\times 100$ , b  $\times 400$ ).

peared in the lower epidermis, somewhat more prominent in one of the patients. In the papillary dermis, a rather small number of lymphocytic cells were accompanied by a varying degree of oedema. In particular, perivascular lymphocytic infiltrates were

consistently found at the lower papillary/upper reticular dermis, with no such infiltrates in the deeper dermis.

Immunocytochemically, the lymphocyte-like infiltrates were a mixture of CD4+ and CD8+ cells. Likewise, the infiltrates

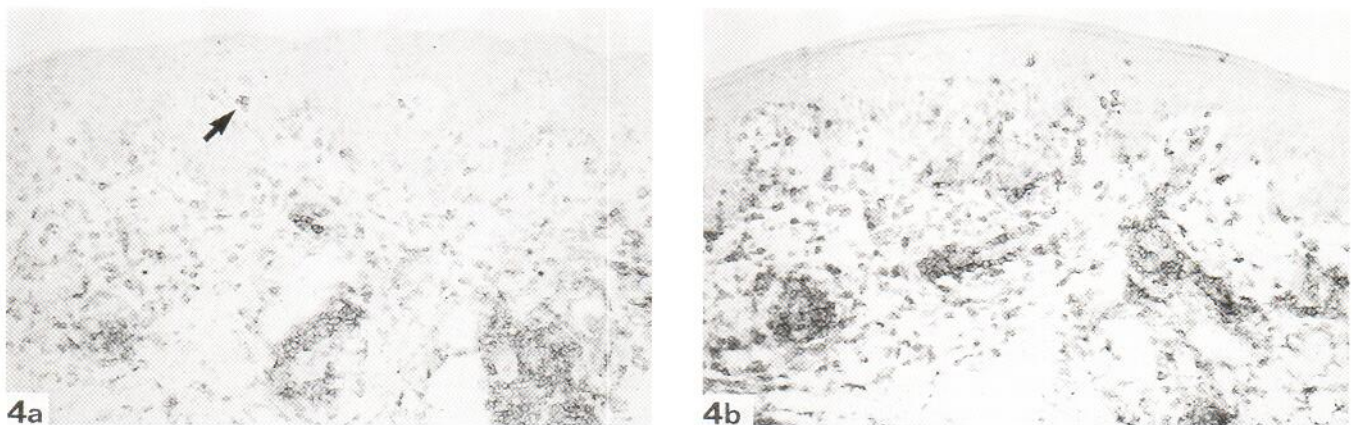


Fig. 4. (a) Clinically positive GTS patch test site (group 2). Frozen section of (a) incubated with UCHL-1 antibody ("memory T"). A significant number of stained cells occur in the papillary dermis, with isolated cells also in the epidermis (arrow). Frozen section of (b) incubated with PD7 antibody ("naive T"), showing a distribution of stained cells similar to (a). (a  $\times 140$ , b  $\times 140$ ).

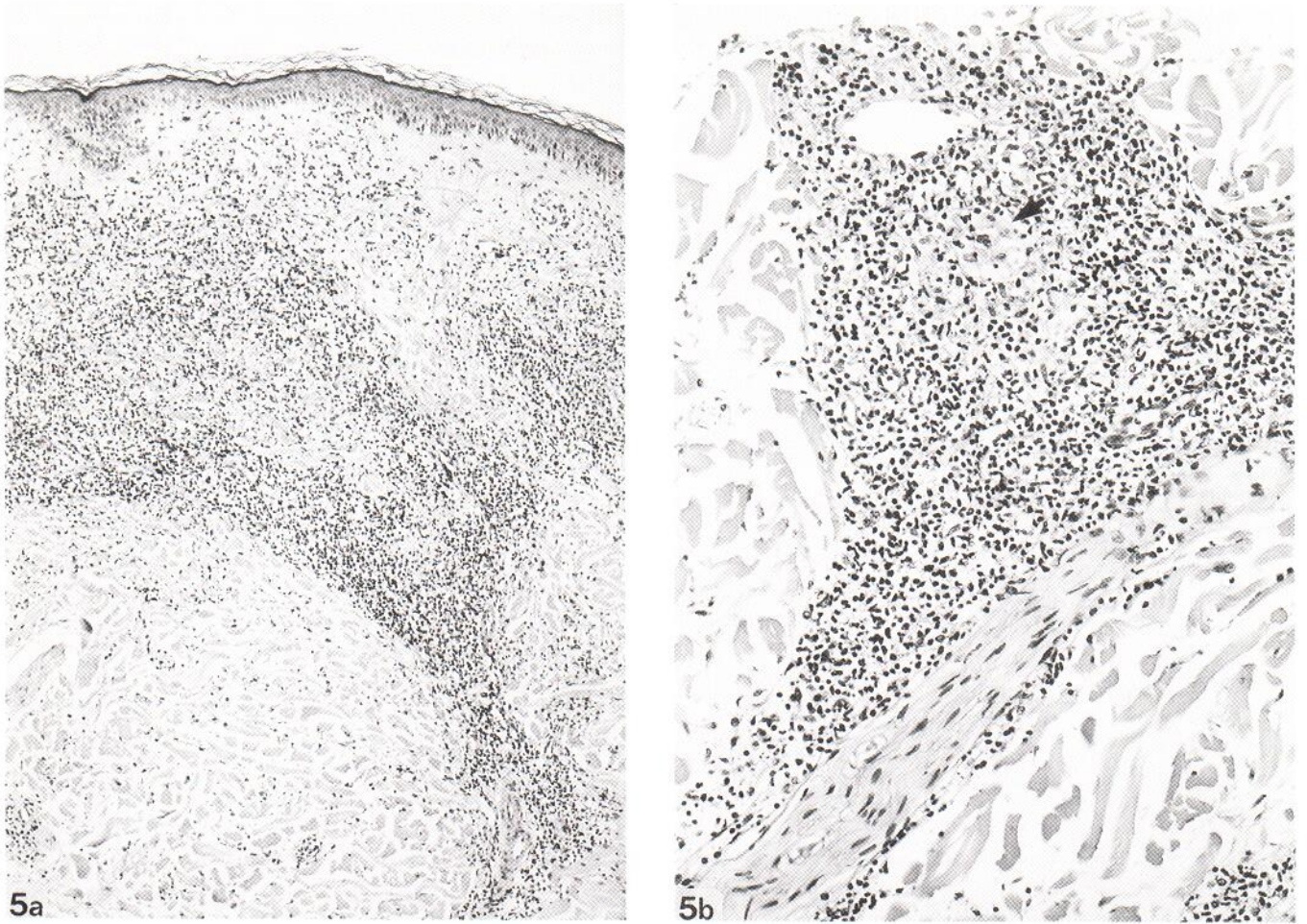


Fig. 5. (a) Long-lasting (10 weeks) positive GTS patch test site (group 4). Papillary dermis is heavily infiltrated by lymphocytic cells, also encroaching upon the reticular dermis. (b) Higher magnification of deeper reticular dermis of (a) shows focal, perivascularly arranged lymphocytic cells, with a tendency to granuloma formation with epithelioid-like cells (arrow). HE-staining. ( $a \times 100$ ,  $b \times 200$ ).

comprised a mixture of "naive" (PD7+ Leu 18+) and "memory" (UCHL1+) cells (Fig. 4), without significant differences between the PD7 and Leu 18 reactions. In the epidermis, stained cells could be demonstrated with all these markers. With CD1a, dendritic cells were stained in the epidermis and isolated cells showed staining in the dermis.

#### Group 3

In patient C, examined 8 h after nickel and GTS application, a very discrete number of PD7+ Leu 18+ and UCHL1+ cells could be seen in both biopsies. The cells appeared at small vessels in the papillary dermis. Associated with these cells were a few OKT6+ dendritic cells as well as HLA-DR cell surface stained cells. In these two 8 h post-application sites, no clear differences could be seen between the two biopsies. In the other 2 patients, biopsied at 4 h after GTS testing, similar small foci of PD7, Leu 18 and UCHL1-stained cells were seen at vessels in the papillary dermis. In patient A, also tested with formaldehyde, it was not possible to clearly detect any difference from the GTS reaction. The same was found in the test reaction to lanolin in patient B. In this patient, the Kathon tested site showed basically the same staining pattern of cells but the stained cells were few

than in the other biopsies. In our test-negative control patient no evidence of focally accumulated lymphocytes was observed.

#### Group 4

One patient was biopsied at 2 weeks after GTS testing. Tissue changes were similar to some of the biopsies of group 1 above, with perivascular lymphocytic infiltrates in the papillary dermis but without deep involvement of the reticular dermis. Sebaceous gland involvement was clearly seen. In a second patient, biopsied at 5 weeks following GTS testing, changes were not clearly different from those of the first patient. In contrast, in a third patient (10 weeks), prominent lymphocytic infiltrates were present throughout the papillary and reticular dermis (Fig. 5a), including sebaceous gland involvement. In some deeply situated areas, the inflammation was granulomatous with epithelioid cells (Fig. 5b).

#### DISCUSSION

A few case reports on contact allergy to gold also contain a histological examination of biopsies taken from the patch test reaction at the regular reading time. We have found five such reports, three dealing with gold trichloride (6–8), one with gold

sodium thiomalate (9), and one with GTS (4). Routine histological preparations have shown an eczematous picture, with exocytosis of mononuclear cells and spongiosis in epidermis, as well as perivascular infiltrates of mononuclears in upper dermis (9). Two reports on gold trichloride also mention features of acantholysis (6, 7).

The present findings show that GTS applied epicutaneously as a patch test seems to cause a consistent pattern of tissue changes in patients showing clinical signs of contact allergy in such a test. T lymphocytes with a mixture of "naive" and "memory"-type T cells (10) appear at a very early stage at small vessels in the lower papillary dermis, consistent with the early stage of a contact allergy (11). Very few cells could be detected at this stage in the epidermis. The perivascular infiltrates gradually increased in size to also involve vessels in the reticular dermis and adnexal organs. Concomitantly, there was evidence of T cell migration into the epidermis, but the extent of spongiotic change was never prominent. This tissue reaction was not found to be significantly different from the nickel-tested sites. However, an additional GTS finding was that test reactions persisted for a long period of time, not known to be a feature of nickel allergy.

A number of studies have been conducted on possible differences between allergic and irritant patch test reactions. In the epidermis, spongiosis or intercellular oedema has been frequently reported as a consistent sign of allergy, occasionally resulting in vesicle formation (12). In the present study, spongiosis was not prominent, neither in nickel- nor in GTS-tested sites. Indirectly, this suggests that the relatively poor degree of spongiosis registered in the GTS sites could still be compatible with an allergic type of reaction. In addition, we found only insignificant intracellular epidermal oedema and no obvious granulocytic component, changes which have often been associated with toxic reactions (12, 13).

Further evidence pointing at an allergic type of reaction at the GTS sites was the finding of oedema in the papillary dermis, accompanied by a gradual increase of mononuclear cells and participation of adnexal organs. These changes seem to have been consistently observed in the dermis of allergic patch test reactions. Thus, "superficial plexus of veins" (14) or "papillary blood capillaries" (12) have been reported to be involved early. On the basis of the findings as regards the other allergens tested in our study, we conclude that the tissue reactions at the GTS-tested sites are highly suggestive of a contact allergy.

Gold drugs have well-known properties as potential anti-inflammatory drugs. However, gold is also the subject of a gradually increasing interest in immune research due to its possible role as inducer of adverse immune reactions (15–17). The pathogenic mechanisms by which gold may cause such effects are unknown. Recent data indicate possible pathways which should also be considered in order to formulate hypothetical explanatory models for the presently observed persistence of the GTS-induced test reactions, also seen in other studies (2–4). It has been postulated that gold (I) may be bio-oxidized to gold (III) in monocytic macrophagic lysosomes (18). Gold III in turn is a potent biooxidizer and may alter the presentation of self-proteins or alter the MHC molecules themselves. This may result in local immune reactions with graft-versus-host-like fea-

tures (18). The development of epithelioid cells may also indirectly support the involvement of tissue macrophages in this process. Thus, the immunogenicity of gold may result in an MHC-associated bioactivity in sensitized individuals, which may partly explain the findings of the present study.

In the present study on patch test reactions of varying strength there was a good correlation between the clinical and histological picture. Even so, in clinically very weak or negative reactions still some lymphocytic cells could be observed in papillary dermis, particularly around superficial vessels. With the present prerequisites, we know that these test reactions were positive although clinically negative. This indicates that many cases of weak contact allergy are probably overlooked due to the insufficiency of the patch test technique.

It is the experience of the present as well as several previous authors that positive patch test reactions to gold salts may persist for weeks and months. Some authors have also described the histological picture of such persistent reactions (4, 19–21). In these cases the dermal lymphocytic infiltrate has predominated, with only slight epidermal changes. In the early reports even a "dermal contact allergy" was suggested. A similar histological picture was observed in our three cases of persistent test reactions to GTS. In one case (Fig. 5), the lymphocytic infiltrate was admixed with epithelioid-like cells with a tendency to granuloma formation. In intracutaneous test reactions to GTS granulomatous changes are not uncommon (to be published). It is interesting that in many cases of clinical contact dermatitis due to gold a histological examination of the diseased skin has revealed a lymphadenoid or pseudolymphomatous structure with practically no epidermal alterations of eczematous character (9, 20, 22–27).

In conclusion, the tissue reactions in positive patch tests with gold and nickel cannot be differentiated by routine histological and immunohistochemical techniques. In other words, the tissue changes induced by GTS are those traditionally observed in contact allergy. We find this important since in most of our cases with positive patch test reactions to gold sodium thiosulfate there was no correlation to a clinical contact dermatitis or stomatitis elicited by metallic gold (5).

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