

# Decreased Trace Element Contents in Chromatin of Patients with Pemphigus

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Three trace elements, manganese, copper and zinc, selected as normal constituents were measured in the chromatin fraction of peripheral blood mononuclear cells from four pemphigous patients and compared with values from 4 matching control cases treated with corticosteroid. Manganese and copper were decreased in pemphigous patients significantly against controls whereas zinc was decreased slightly without statistical significance. Decreased trace element levels in chromatin revealed a unique pattern characteristic only for pemphigus as compared with earlier data of this group and of others in different pathological states. Decrease in chromatin zinc content might reflect the fact of corticosteroid treatment rather than a pathognomic feature which has to be taken into consideration while working with samples from patients on long-term steroid therapy. The theory of trace element depletion in pemphigus is confronted with known reports of penicillamine—a chelating agent—induced cases of the disease. *Key words: Manganese; Copper zinc; Lymphocytes; Pemphigus; Interphase chromatin.* (Received April 14, 1983.)

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In recent years much attention has been devoted to the aetiopathogenesis of pemphigus (9). Drugs of the penicillin group, especially penicillamine were made responsible for the induction of antiepithelial antibodies which in turn could induce intracellular protease (s) manifesting in symptoms of pemphigus (4, 9).

The aim of these studies was to investigate the trace elements in the chromatin of pemphigous and control patients while penicillamine was used as chelating agent to deplete heavy metals some of whose being also currently investigated, in pathological conditions (4).

Chromatin was chosen because several trace elements in particular manganese copper and zinc are its normal constituents and they might play a significant part in the maintenance or change of its tertiary and quaternary structure and, presumably, in its function.

## METHODS AND MATERIAL

With the help of a Ficoll-Urografin technique mononuclear cells (approx. 85% lymphocytes, 15% monocytes) were separated from the blood of patients with pemphigus and from that of control subjects. Cells were washed in phosphate buffered saline containing glucose (PBSG) and resuspended to the final cell count of  $1.0 \pm 0.3 \times 10^7$  per ml. They were then sedimented by centrifugation and lysed in 5.0 ml 'lysis solution' (0.1 mol/l EDTA, 2 mmol/l Tris-HCl pH 7.8, 0.5% Triton X-100) at room temperature for 5 min. This was followed by homogenisation for 1 min at 4°C in an IKA cell homogeniser (Janke & Kunkel, GFR).

Then the total lysate was transferred into plastic vials suitable for neutron activation analyses. The crude chromatin was separated by centrifugation at 4°C, 3 000 g. The sediment was washed by 3.0 ml of PBSG and termed "chromatin". This fraction contained 95% of the total cellular DNA and 15% of

Table 1a. Mn, Cu and Zn concentration in chromatin of mononuclear cells from the peripheral blood of pemphigous patients

Pat. no.	Sex	Age (yrs.)	Duration of disease	Total corticost. dose (mg)	Present corticost. dose (mg)	Mn (ng/mg P)	Cu (ng/mg P)	Zn ( $\mu$ g/mg P)	Diagnosis
1	Woman	32	9 mo.	Approx. 13.000	45 mg methylprednisolone/day per os, 2x40 mg triamcinolone acetonide per week, 12 hrs. after last inj.	114	224	2.9	Penicillin induced pemphigus
2	Man	53	5 yrs.	More than 50.000	2x5 mg dexamethasone/day iv, 12 hrs. after last inj.	43	425	6.9	Seborrheic pemphigus, diabetes mellitus, glaucoma, icterus cholestaticus
3	Woman	70	8 yrs.	More than 50.000	20-40 mg prednisolone per os on alternate days, 24 hrs. after 20 mg	138	268	57.5	Pemphigus vulgaris, diabetes mellitus, hypertonia
4	Man	83	1 yr.	Approx. 5.000	40 mg methylprednisolone/day per os	121	274	55.9	Pemphigus vulgaris, cachexia, bronchopneumonia
Mean $\pm$ SD		59.5 $\pm$ 38.2				93 $\pm$ 43	298 $\pm$ 88	38.8 $\pm$ 31.1	
<i>p</i>		NS				0.065	0.049	NS	

the total cellular protein. The inorganic phosphorus content was about 10  $\mu$ g per 10<sup>6</sup> cells. The vials were sealed and irradiated for 30 min in the ASTRA reactor with a thermal neutron flux of  $7 \times 10^{13}$  n/cm<sup>2</sup> (1). Four hours after neutron activation Mn<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup> were measured with a Canberra 4000-channel pulse height analyser which connected to an 85 cm<sup>3</sup> Ge/Li-detector (resolution 1.7 keV). Phosphorus contents were measured as neutron activated <sup>32</sup>P after three weeks on the basis of the Cerenkoff effect in a liquid scintillation spectrometer (model 3375 Packard, USA).

**P Standards:** Standards for neutron activation analysis were prepared from solution containing 20  $\mu$ g of pure P as (NH<sub>4</sub>) H<sub>2</sub>PO<sub>4</sub> (P. A. Merck Chemical Company). Five  $\mu$ l from (NH<sub>4</sub>) H<sub>2</sub>PO<sub>4</sub> solution were pipetted on filterpaper (SELECTA, Schleicher & Schüll, No. 595) put in polyethylene container and closed. Accuracy and procession was about  $\pm$ 2% in range 1–20  $\mu$ g P.

**Cu, Zn, Mn Standards:** Standards for neutron activation analysis were prepared from Titrisol solution (P. A. Merck, Art. No. 9987, 9953, 9988) containing 20  $\mu$ g pure Cu, 100  $\mu$ g pure Zn, 5  $\mu$ g pure Mn

20  $\mu$ liter from Titrisol solution for Cu

100  $\mu$ liter from Titrisol solution for Zn

5  $\mu$ liter from Titrisol solution for Mn

were pipetted on filterpaper (SELECTA, Schleicher & Schüll, No. 595) put in polyethylene container and closed. Accuracy and procession was about

$\pm$ 5% in range 1–20  $\mu$ g Cu,

$\pm$ 10% in range 10–100  $\mu$ g Zn, and

$\pm$ 5% in range 1–5  $\mu$ g Mn.

Statistical analysis of results was carried out using the non-paired *t*-test.

Table 1 b. *Mn, Cu and Zn concentration in chromatin of mononuclear cells from the peripheral blood of control patients*

Pat. no.	Sex	Age (yrs.)	Duration of disease	Total corticost. dose (mg)	Present corticost. dose (mg)	Mn (ng/mg P)	Cu (ng/mg P)	Zn (µg/mg P)	Diagnoses
5	Man	51	10 days	700	30 mg prednisolone/day per os 24 hrs. after last dose	596	1 605	47.6	Acute penicillin allergy, Quincke's edema, pulmonary emphysema, coronary sclerosis
6	Woman	74	7 yrs.	More than 20.000	10 mg prednisolone/day per os 24 hrs. after last dose	491	2 377	39.2	Pyoderma gangrenosum, erysipelas cruris, glaucoma
7	Woman	37	3 mo.	300	15 mg prednisolone/day per os 24 hrs. after last dose	67	798	7.2	Erythema nodosum
8	Woman	53	3 yrs.	220	None, administr. 4 days before test discontinue	320	531	117.9	Acute wheal due to balsam of Peru, polysensitisation to different chemicals and drugs
Mean±SD		53±26.4				369 ± 231	1 328 ± 835	53.0 ±46.7	

## CASE REPORTS

Four in-patients of the Dermatology Department, Budapest, with pemphigus, 2 males and 2 females were included in the patient group. The disease was diagnosed upon detection of Tzanck cells from the initial lesions, Nikolski phenomenon, acantholysis and cleft formation in histological preparations and positive direct or indirect immunofluorescence in demonstration of antiepithelial antibodies. Four other inpatients (1 male and 3 females) with variable diseases were selected into the control group (Table I.)

All controls received significant amounts of corticosteroid preparations when (3 out of 4) or just before (1 out of 4) the test was made in order to match with the pemphigous group in respect of the treatment. Cytostatic drugs were given to neither pemphigous nor control patients at the time of these studies.

## RESULTS

It is evident from Table I, that while manganese and copper contents decreased significantly among pemphigous against control patients there was no such difference in the average zinc values between the two groups. All three trace elements, however, gave lower readings in the chromatin of pemphigous cells. The two groups of patients could be compared in spite of small sample size because of the close average ages, chronicity of their diseases and previous and present corticosteroid treatments. One patient of the pemphigous group (no. 4) died two days later because of bronchopneumonia and subsequent cardiorespiratory insufficiency. Another patient of this group (no. 1) died one month

later because of septicaemia. The relatively high zinc value is remarkable in the control patient no 8, who finished corticosteroid treatment four days before our test.

## DISCUSSION

Manganese copper and zinc are trace elements and manifest themselves in the form of divalent cations belonging to the 'first transition series'. That means that the stability constants of their complexes formed, e.g. with proteins or nucleic acids are  $>10^{12}$ .

This implies high binding strength. While low binding strength permits exchange, high binding strength does not. Zinc is at the extreme end of this series and behaves differently, its levels change more rapidly than those of copper and manganese.

There is no correlation between the tissue and serum concentration of zinc (6). Metal ions bound to chromatin are of importance in various enzymatic reactions within the chromatin, for instance in the transfer of genetic material or the degradation of nucleic acids. In synchronised eukaryotic cells  $^{14}\text{C}$  guanine incorporation into rapidly labelled RNA and  $\text{Zn}^{2+}$  uptake behave parallel (2). Whereas copper and zinc are bound in nucleic acids preferably to bases, manganese is bound between the  $\text{N}_7$  of a guanine and the phosphate groups.  $\text{Mn}^{2+}$  seems to act on the condensation of chromatin, a process which is regulated by histone  $\text{H}_1$ . Moreover,  $\text{Mn}^{2+}$  together with  $\text{Ca}^{2+}$  can activate intracellular neutral proteases that are located in the cytoplasm. Chromatin bound copper can act as radical scavenger and inactivate  $\text{O}_2^-$  as well as  $\text{OH}^\cdot$  radicals that would act as mutagens.

Our results seem to indicate the lowest amounts of manganese and copper in chromatin among pathological conditions investigated so far. Earlier, Karimian-Teherani et al. (5) reported slightly increased  $\text{Mn}^{2+}$  and  $\text{Cu}^{2+}$  contents and marginally decreased  $\text{Zn}^{2+}$  contents in chromatin of lymphocytes in 17 patients with rheumatoid arthritis. The differences, however, were not significant against controls. The control group of that study consisted of 12 healthy subjects. Their trace element readings did not differ significantly from those of the present control group except for zinc. The zinc value of controls calculated as  $\mu\text{g}/\text{mg}$  chromatin phosphorus ( $53 \pm 46.7$ ) in this study was less than one third of those healthy, untreated controls ( $169.68 \pm 47$ ) (for data see ref. 5).

This difference might be attributed to the corticosteroid treatment even in the control group of the present study. Furthermore, the fact, that the highest zinc level in chromatin was measured in case no 8 of the control group whose corticosteroid treatment was finished before the test has been performed, still supports this view. Therapeutic corticosteroid levels act as suppressors of RNA synthesis while the latter has restored parallel with zinc uptake (2).

In another study of our group the lymphocyte chromatin of 6 patients with multiple warts has shown no significant alterations in  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$  contents but a tendency of increased  $\text{Cu}^{2+}$  levels against 8 healthy controls. Parkinson et al. found in skin biopsies significant increase of manganese and copper in benign nevi and malignant tumours which correlated well with the clinical picture. Zinc determinations gave inconsistent findings (8).

The fact, that the cellular chromatin of pemphigous patients has been depleted of manganese and copper, can be interpreted as a sign of destabilisation in immunologically competent cells. This might be a reflection of lowered suppressor lymphocyte function in pemphigus (3), a condition that is common with systemic lupus erythematosus (7). Another explanation, merely on a speculative level, would say that unique finding of decreased trace elements in pemphigus might be related to the origin of disease. This is supported also by earlier findings that the pemphigous skin contained significantly less  $\text{Ca}^{2+}$  than both that of the normal and of otherwise diseased subjects (10). All these latter data would explain why chelating agents such as penicillamine could induce pemphigus under certain circumstances.

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