

From the Department of Dermatology (Chairman: Prof. W. Jadassohn) and
Laboratory of Prof. hon. E. Bujard (School of Medicine)
University of Geneva, Switzerland.

EXPERIMENTAL ECZEMA¹

(23rd Communication)

Eosinophilic Leucocytes in the Guinea Pig Sensitized to Citraconic Anhydride

By

N. HUNZIKER

In a previous work we examined histologically the immediate and the delayed reaction obtained with guinea pigs sensitized to citraconic anhydride (CA) (*Hunziker*). It was shown that infiltrates had a large number of eosinophilic leucocytes in both reactions.

These results have induced us to make a series of blood smears to evaluate the number of eosinophils in guinea pigs sensitized to CA.

The technique of sensitization is described in a previous report. (*Hunziker*).

In order to count the percentage of eosinophils, the blood was taken from the ear.

Biopsies were made fourteen hours after the application of the epicutaneous test (one application of CA 25 per cent in dioxane).

RESULTS

It was found that the majority of guinea pigs sensitized to CA showed more eosinophils in the blood than do non-sensitized guinea pigs (Table 1).

¹ This work profited by a subsidy from the Swiss National Fund for Scientific Research.

TABLE 1

*Number of Guinea Pigs Presenting an Eosinophilia Greater than
3 per cent in the Blood.*

Non-sensitized guinea pigs	16/128	12 %
Guinea pigs sensitized to CA*	14/18	78 %
Guinea pigs sensitized to DNCB* ..	3/28	9 %

* Examination made 2 to 4 months after the end of the sensitization.

The eosinophil content in the blood is directly related to the eosinophil content in the infiltrate due to the epicutaneous test. The coefficient of correlation is significant ($p < 0.05$).

After frequently repeating blood examinations for several months we found that the number of eosinophils remains more elevated in the sensitized animals than with the control animals of the same weight. The epicutaneous tests also remain positive and always contain numerous eosinophils.

An epicutaneous test to CA on guinea pigs sensitized to this chemical provokes a considerable drop in blood eosinophilia after 1-4 hours and a new increase after 12 to 14 hours.

This new increase often surpassed the number of blood eosinophils counted before the test. The reduction in the number of eosinophils is significant ($p < 0.01$) and the increase in the number of eosinophils is equally significant ($p < 0.05$).

With guinea pigs sensitized to CA we frequently found an increase of eosinophils in the skin of non-treated regions but not in non-sensitized guinea pigs (Table 2).

With guinea pigs sensitized to dinitrochlorobenzene (DNCB), we did not note an elevation in the number of eosinophils in the blood (Table 1), nor in the infiltrate provoked by epicutaneous tests.

We successively sensitized guinea pigs to CA and to DNCB. The epicutaneous tests to DNCB, which were positive, showed more eosinophilic leucocytes in the infiltrate than with animals sensitized only to DNCB and tested in the same manner. The number of eosinophils is *higher* than in the non-treated skin of guinea pigs sensitized to CA, but *lower* than in the skin of guinea pigs sensitized to CA and tested with CA. It will be

remembered that animals sensitized to CA and DNCB had a blood eosinophilia which is not found in animals sensitized only to DNCB.

With guinea pigs sensitized to CA, an intraperitoneal injection of hydrocortisone disuccinate (25 mg per guinea pig of 300–400 g) produced a regular fall in the number of eosinophilic leucocytes in the blood to 0–0.5 per cent in the 6–8 hours. Only 3–7 days after the injection of hydrocortisone disuccinate does the initial eosinophilia in the blood return.

TABLE 2
Eosinophilic Leucocytes in the Skin of CA sensitized Guinea-Pigs without a Preliminary Test.

Guinea pigs sensitized to citraconic anhydride	Eosinophils in the skin	Non-sensitized guinea pigs	Eosinophils in the skin
209	+	654	—
157	±	655	—
202	±	656	—
298	±	657	—
344	++±	464	—
319	±	565	—
613	+±		
623	±		

— : absense of eosinophils or very slight presense.

± : several eosinophils.

+ and +± : numerous eosinophils.

++ and ++± : large number of eosinophils.

With guinea pigs sensitized to CA we applied an epicutaneous test with CA at the moment the eosinophil count fell to 0–0.5 per cent due to the injection of hydrocortisone disuccinate. The tests were macroscopically positive. We conducted a biopsy of the tests after 14 hours, when there was a low eosinophilia. In the infiltrate of the test, we noted numerous eosinophils: intravascular (Fig. 1), perivascular and disseminated in the dermis.

With guinea pigs sensitized to CA, that were injected with hydrocortisone, we found eosinophilic leucocytes, sometimes

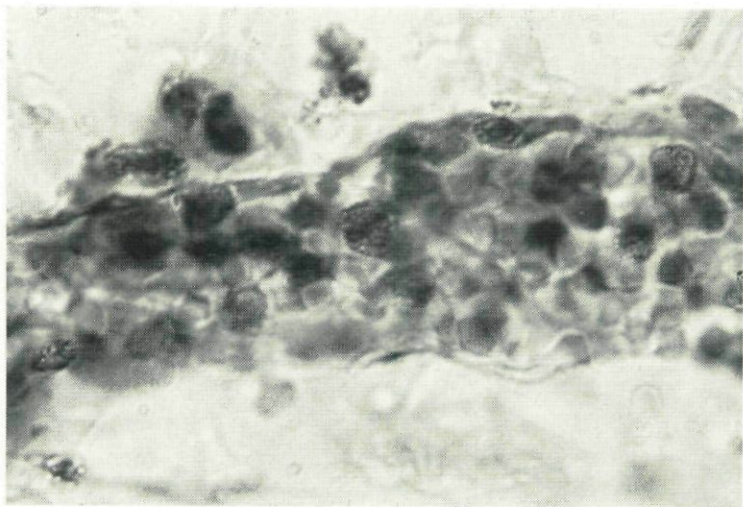


Fig. 1.

Eosinophilic leucocytes in a blood vessel of a citraconic anhydride tested guinea pig after hydrocortisone injection. (Immersion).

quite numerous in the dermis of the skin in the non-tested regions; perhaps even more than with guinea pigs sensitized to CA but not having received hydrocortisone.

DISCUSSION

We have observed that with guinea pigs sensitized to CA, numerous eosinophils are seen in the infiltrate of the dermis after an epicutaneous test and also eosinophil leucocytes are increased in the blood of these animals.

There is a parallel between the blood eosinophilia and the contents of the eosinophilic leucocytes in the dermal infiltrate. The eosinophilia in the blood like the sensitivity persists for months when the animals are tested from time to time.

We previously reported that the late reaction in animals sensitized to CA was not distinguishable macroscopically from that of the animals sensitized to DNCB. We found at the level of the nipple some spongioses, like that found in animals sensitized to DNCB. The animals sensitized to CA differ

from animals sensitized to DNCB by a urticarial reaction which precedes the late reaction, by the fact that the late reaction to CA was passively transferred by the serum, by the eosinophilia in the blood, and numerous eosinophils in the infiltrate of the tested skin which did not exist in animals sensitized to DNCB.

The presence of numerous eosinophils in the infiltrate of epicutaneous tests in guinea pigs sensitized to CA suggests two possibilities:

a) the eosinophils are of blood origin (hematogenic) and come from an emigration in the dermis.

b) the eosinophils are of local origin (histiogenic), as is thought by *Dzury* and *Cohen*: "Local tissue eosinophils of the skin may have a source of origin independent of peripheral blood eosinophils".

In 1932, *W. Jadassohn* had already supposed that the eosinophils were attracted to the injection point by a urticariogenic agent, that they fixed themselves at the level of the urticariogenic reactions, and by this process were removed from the blood circulation; they were over compensated in the blood afterwards by the bone marrow. The author stressed, however, that this was only an hypothesis; as did *Lang & Berger*.

A study by *Hofer & Golay* conducted in our clinic showed that in two patients having received cortisone for 4 days and in which there was 0 to 1.5 per cent eosinophils in the blood, there was a clear reduction in the number of eosinophils in the infiltrate of the urticarial papule. On the other hand, the cortisone had not had an influence on the number of eosinophils in the infiltrate in another case where the number of blood eosinophils was not lowered by cortisone.

In favor of the hypothesis for a hematogenic origin of the eosinophils of the infiltrate are the following observations:

1) With guinea pigs sensitized to CA there exists an eosinophilia in the blood which is directly related to the large number of eosinophils at the level of epicutaneous tests.

2) One hour after the application of the test with CA on guinea pigs sensitized to this substance, there was a reduction of the number of eosinophils in the blood followed by an increase.

3) Guinea pigs successively sensitized to CA and to DNCB present a blood eosinophilia due to the sensitization to CA. If these guinea pigs are tested with 0.1 per cent DNCB, the infiltrate as the level of the epicutaneous test contains more eosinophils than in guinea pigs sensitized only to DNCB and which do not show an increase in blood eosinophils.

However, with guinea pigs having a low blood eosinophilia after injection of hydrocortisone, numerous eosinophilic leucocytes are seen at the level of the epicutaneous tests, some eosinophiles in the dermis, but equally in the small vessels (Phot.). It seems probable that a lowering of the eosinophil number in the blood to 0.5 and 0 per cent does not signify a total absence of blood eosinophils in the circulation, and these remaining eosinophils are stopped at the level of the tests.

We believe that our observations speak in favor of the mechanism discussed by *W. Jadassohn* and *Berger & Lang* for the urticarian reactions. Eosinophils of blood origin are stopped locally, either by an urticariogenic agent or, as in our work, by citraconic anhydride.

The hypothesis formulated in 1932 by *W. Jadassohn* is now much better founded than at that time. Nevertheless it must be examined further.

Guinea pigs sensitized to CA with blood eosinophilia lend themselves towards numerous examinations concerning problems posed by these cells in the blood and in the tissues. Our verification that some eosinophils are located in the skin of non-tested regions in guinea pigs sensitized to CA merits closer examination. We have even found eosinophils in large numbers in the non treated skin of CA sensitized animals, which had a low blood eosinophilia due to hydrocortisone.

Besides these problems, we can also consider employing the CA sensitized guinea pig, with its blood eosinophil increase,

in the examination of practical problems such as the replacement of hydrocortisone disuccinate employed in our experiments by other derivatives of cortisone or by "cortisone-like" substances.

SUMMARY

Sensitization to citraconic anhydride (CA) in guinea pigs provokes an eosinophilia in the blood. Epicutaneous application of CA diminishes this blood eosinophilia. Hydrocortisone injections provoke a very strong blood eosinophil drop (0-0.5 per cent) ! An epicutaneous CA test applied during this "eosinopenia" in the blood shows, in spite of the "eosinopenia", many eosinophil leucocytes in the dermis. Our results suggest that the eosinophils localised under certain circumstances in the dermis, are of hematogenous origin.

REFERENCES

- Berger, W. & Lang, F. J.*: Zur Histopathologie der idiosynkratischen Entzündung in der menschlichen Haut. Beitr. path. Anat. 87, 71, 1931.
- Dzury, D. S. & Cohen, Sh. G.*: Experimental eosinophilia. Studies on the origine and relationship of tissue eosinophil cells to peripheral blood eosinophilia. J. Allergy, 29, 340-357, 1958.
- Hofer, R. & Golay, M.*: Action de la cortisone sur la papule urticarienne provoquée par la morphine. Dermatologica, 116, 197-200, 1958.
- Hunziker, N.*: De l'eczéma expérimental. 20ème comm. Réactions du cobaye sensibilisé à l'anhydride citraconique. Dermatologica, 120, 89-100, 1964.
- Jadassohn, W.*: Eosinophile und urticarielle Quaddel. Arch. f. Derm. u. Syph. 166, 458-462, 1932.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.