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BASOPENIA IN URTICARIA

By

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In many cases of urticaria there is a decrease in the number of basophil leucocytes in the circulating blood (*Rorsman* 1957; *Rorsman & Rosengren* 1958; *Bersani* 1959; *Meneghini & Levi* 1958 and 1959; *Levi, Meneghini & Rantuccio* 1959). This decrease may be explained in different ways. The following possibilities can be considered:

1. Decrease in the formation of basophils in the bone marrow.
2. Decrease in the outflow of basophils from the bone marrow.
3. Destruction of basophils at the site of their formation, in the circulation or outside the blood vessels.
4. Degranulation of basophils which would make it impossible to identify them with the methods used by the above-mentioned authors.
5. Trapping of basophils in any tissue (and then most probably in the diseased skin).

Changes according to any of these alternatives could be due to the agent causing the urticaria, or to substances formed or released in the whealing tissue. There is evidence of release of histamine and of heparin-like substances in urticaria (*Nilzén* 1947; *Adam, Hunter & Kinnear* 1950; *Asboe-Hansen* 1950 and 1951), and these compounds might directly or indirectly influence the basophils, for example, via adrenal stimulation.

The aim of this investigation is to obtain information on the mechanisms responsible for urticarial basopenia.

The following studies have been made:

Examination of basophils in smears from sternal bone marrow in cases of urticaria.

Examination of blood smears from peripheral blood in cases of urticaria, attention being paid particularly to totally or partly degranulated basophils.—This study was preceded by a study of the nuclear morphology of basophils in normal persons in order to find out if the nuclei had characteristics allowing diagnosis of the cells irrespectively of metachromatic granulation.

Incubation of basophils in hypotonic solutions to find out if swelling of basophils could be observed prior to loss of granules, thus indicating imminent degranulation of the cells.—This study was made after some experiments on degranulation of basophils *in vitro*, in which we found signs of partial degranulation though this was very difficult to quantitate. As swelling of tissue mast cells precedes degranulation (Uvnäs & Thon 1959) it was thought that swelling of basophils might be a more distinct sign of cell damage than degranulation. Hypotonic solutions lent themselves to testing if the metachromatic granules of basophils could at all be preserved at an observable swelling of the cells.

Incubation of basophils in heparin- and histamine-solutions to find out if these substances influenced the basophil count *in vitro*. Injected heparin or histamine may decrease the number of basophils *in vivo* (Braunsteiner & Thumb 1958; Leo 1956) and endogenous heparin or histamine might cause urticarial basopenia.

Incubation of normal basophils with sera from patients with urticarial basopenia to find out if these sera had degranulating properties demonstrable *in vitro*.

Biopsies from wheals in patients with urticaria for examination with regard to possible local accumulation of basophils.

Determinations of the excretion in urine of 17-KS and 17-KGS in patients with urticaria.—An increased excretion of

adrenal steroids has been observed in urticaria (*Roskam, van Cauwenberge & Lecomte* 1956), and ACTH and corticosteroids are known to decrease the number of circulating basophils (*Torii et al.* 1953; *Code, Mitchell & Kenedy* 1954; *Osada* 1954; *Kelemen & Bikich* 1956; *Noah & Brand* 1957; *Tatai & Osada* 1957; *Boseila & Uhrbrand* 1958; *Thonnard-Neumann* 1958 and 1961; *Braunsteiner & Thumb* 1958).

MATERIAL AND METHODS

The patients with urticaria and psoriasis were treated at the Department of Dermatology at Lund.

The healthy subjects were students or members of the clinic staff at the Lund Department of Dermatology or at the Malmö Department of Epidemiology.

Sternal bone marrow was examined in 11 patients with urticaria, eight males and three females, aged between 17 and 61; in six healthy persons,¹ all males, aged between 20 and 59; and in eight patients with psoriasis, six males and two females, aged between 17 and 67.

Morphology of basophil nuclei in peripheral blood was studied in 20 healthy persons, eight males and twelve females, aged between 22 and 59.

Basophils in smears of peripheral blood was studied in twenty cases of urticarial basopenia, eleven males and nine females, aged between 17 and 59.

Incubation experiments were performed with basophils from healthy persons.

The effect of hypotonic solutions on basophils was examined in cells from ten persons, six males and four females, aged between 23 and 59.

The effect of heparin on basophils was examined in cells from five persons, three males and two females, aged between 24 and 31.

The effect of histamine on basophils was examined in cells from five persons, two males and three females, aged between 26 and 42.

The effect on basophils of serum from patients with urticarial basopenia, was examined in cells from ten healthy persons, five males and five females, aged between 17 and 52. Serum was obtained from ten patients with urticarial basopenia, five males and five females, aged between 26 and 62, and from ten healthy persons, five males and five females, aged between 24 and 59.

Biopsies of wheals were performed in 15 urticaria patients, six males and nine females, aged between 13 and 60.

Excretion of neutral 17-ketosteroids (17-KS) and of 17-ketosteroidogenic steroids (17-KGS) was determined in 15 patients with urticaria without fever, five males and ten females, aged between 17 and 61.

Direct basophil count was performed in all subjects studied with the technique

¹ Dr. H. Hellsten and Dr. S. Belfrage gave me kind permission to study this material.

of Rorsman (1957), which is a modification of the technique of Moore & James (1953).

Sternal *bone marrow smears* were air-dried and stained with May-Grünwald-Giemsa or according to Undritz (1950). For the myelogram 400 white cells were differentiated. Basophil count was based on examination of 10,000 white cells.

The *morphology of basophil nuclei* was studied in smears stained according to Undritz (1950), which after identification of the basophils were decolourized in N hydrochloric acid for two hours and then restained for four minutes in Giemsa solution after fresh fixation in methyl alcohol for four minutes. This procedure was necessary as the metachromatic granules in simple basophil staining generally cover the nucleus.

Basophils in smears from peripheral blood were stained according to Undritz (1950). Basophils per 2000 leucocytes were examined in each patient and *indirect basophil count* was based on differential count of 2000 leucocytes, and on total leucocyte count performed with the technique described by Rorsman & Rosengren (1958).

The *incubation experiments* were performed with citrated blood (the experiments on the effect of hypotonic solutions, heparin and histamine) or with a leucocyte suspension obtained from citrated blood by aggregation of the erythrocytes with dextran (the experiment on the effect of serum on basophils).

Citrated blood: 8 ml venous blood, mixed with 2 ml 0.1 N sodium citrate solution.

Leucocyte suspension: 10 ml citrated blood, mixed with 2 ml saline containing 6% dextran, prepared by AB Pharmacia, Sweden (Fraction TDR 205-II-B-1, intrinsic viscosity = 0.40, molecular weight about 300,000) was allowed to stand for 30 minutes. After this time the erythrocytes were sedimented and the plasma containing the leucocytes could be pipetted off. The plasma was centrifuged for 10 minutes at 1,000 rpm in a Corda centrifuge with a diameter of 30 cm. After pipetting off plasma to 1 ml and two washings in 9 ml isotonic phosphate buffer, pH 7.4, the leucocyte suspension was used for serum incubation experiments.

All glass-ware was siliconed.

Direct basophil counts were made after incubation in air at 37° C for 30 minutes. The incubation arrangements were as follows:

Hypotonic solution: To each 1 ml citrate-blood was added 9 ml of a solution containing isotonic phosphate buffer pH 7.4 and distilled water in the following proportions: 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, or 1:9. Control: 1 ml citrate-blood and 9 ml isotonic phosphate buffer pH 7.4. Centrifugation and pipetting off to 1 ml as of leucocyte suspension.

Heparin: To each 0.9 ml of citrate-blood was added 0.1 ml of a saline solution containing 25, 2.5, 0.25 or 0.025 mg heparin/ml. No preservative was added to the heparin. Control: 0.9 ml citrate-blood and 0.1 ml saline.

Histamine: To 0.9 ml of citrate-blood was added 0.1 ml of a saline solution containing 1 mg histamine-dihydrochloride/ml. Control: 0.9 ml citrate-blood and 0.1 ml saline.

Serum: To 0.5 ml leucocyte suspension was added 0.5 ml serum from a patient with urticarial basopenia. Control: 0.5 ml leucocyte suspension and 0.5 ml serum from a person with a normal basophil level.

Biopsies from wheals and from symmetric skin without wheals were made at the same time with a punch 7 mm in diameter without anesthesia.

The skin specimens were fixed for 24 hours in a solution of 4 per cent basic lead acetate which was freshly prepared. After this, a fixation in 4 per cent formaldehyde solution was also performed for 24 hours. The specimens were then dehydrated, embedded in paraffin and sectioned at 10 μ . Staining was made in 0.1 % toluidine blue (Merck) in 0.01 N acetate buffer, pH 5, for 5 minutes. After passing through increasing concentrations of ethyl alcohol and xylol the sections were mounted in Canada balsam. A piece from each specimen was fixed only in 4 per cent formaldehyde solution and stained in hematoxylin-eosin.

Determination of neutral 17-ketosteroids (17-KS) and of 17-ketosteroidogenic steroids (17-KGS) was performed on urine collected for 24 hours in bottles containing 2 ml chloroform and 8 ml ethyl alcohol. The determination of steroids was performed at Hormonlaboratoriet, Kvinnokliniken, Allmänna Sjukhuset, Malmö.¹ The method used for 17-KS was that described by Genell, Jensen & Lindegård (1956) and for 17-KGS that of Appleby, Gibson, Norymberski & Stubbs (1955).

RESULTS

Bone marrow examination of basophils.

The basophil findings in marrow smears from 11 patients with urticaria are given in table 1. The number of basophils is higher in the bone marrow than in the peripheral blood. Comparison of basophil findings in the bone marrow of urticaria patients with the findings in normal persons and in psoriasis patients with normal basophil level (table 2), shows that the per cent basophil count in the bone marrow is not decreased in urticaria in spite of peripheral basopenia. The basophil nucleus is generally covered by metachromatic granules, so it is only occasionally possible to state definitely the character of a basophil cell. It is possible, however, to distinguish between basophil leucocytes and tissue mast cells, which was seen in the bone marrow smears of urticaria-case 11 (Fig. 1). In most cases the tissue mast cells have a more irregular form than the round

¹ Dr. C. Jensen kindly discussed the results and gave me permission to use the normal distribution curves.

TABLE 1
Basophils in the bone marrow of urticaria patients.

Patient no.	Age	Sex	Leucocytes per mm ³ peripheral blood	Basophils per mm ³ peripheral blood	Basophils per 10,000 white cells in bone marrow
1	49	F	4,000	0	22
2	46	M	5,400	0	8
3	22	M	5,200	15	54
4	17	F	3,800	0	14
5	40	M	3,800	0	59
6	54	M	7,000	1	89
7	61	M	6,300	5	33
8	42	F	5,600	2	30
9	28	M	6,200	2	16
10	17	M	7,900	0	57
11	29	M	7,900	0	46*

* Several tissue mast cells in the marrow smear.

TABLE 2
Basophils in the bone marrow of healthy persons and of patients with psoriasis, all with normal basophil counts in peripheral blood.

Subject no.	Diagnosis	Age	Sex	Leucocytes per mm ³ peripheral blood	Basophils per mm ³ peripheral blood	Basophils per 10,000 white cells in bone marrow
1	Healthy	49	M	5,100	32	16
2	"	25	M	6,200	21	29
3	"	59	M	3,900	26	31
4	"	24	M	6,100	49	42
5	"	44	M	4,800	29	41
6	"	29	M	5,000	43	17
7	Psoriasis	34	M	4,500	41	30
8	"	32	M	6,400	26	53
9	"	25	F	5,100	34	31
10	"	51	M	10,200	61	22
11	"	17	F	4,600	37	71
12	"	43	M	9,800	41	35
13	"	67	M	4,300	23	26
14	"	35	M	6,700	67	41

basophil leucocytes. If the tissue mast cell is round it may be differentiated through the *finer granules*, mainly *located at the periphery of a round or oval nucleus*. The differences between tissue mast cells and myeloid metachromatic cells in smears were first pointed out by *Moeschlin* (1947).

There was no remarkable change in the count of other blood cells in the bone marrow of urticaria patients.



Fig. 1.

Tissue mast cell in human bone marrow. $\times 2600$.

Morphology of basophil nuclei.

As stated previously, the metachromatic granules of basophil myeloid cells make it difficult to observe the nucleus. In Giemsa stained basophils, identified before the decolourizing of granules, it was found that the segmentation of the nuclei was similar to that of other granulocytes, although the nuclear segments were often closer to each other than in other granulo-

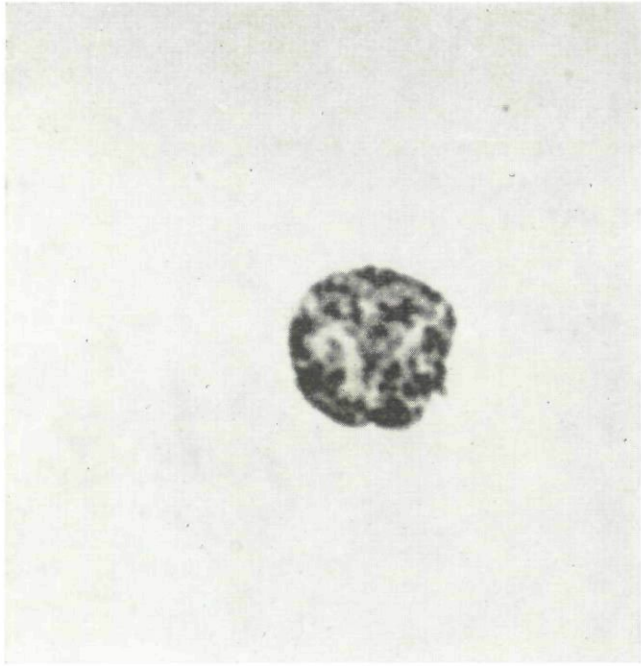


Fig. 2.

Basophil leucocyte in peripheral blood, stained according to Undritz.
The nucleus can hardly be observed. $\times 2600$.

cytes. The chromatin often appeared more compact in the basophils than in other leucocytes. The nuclear morphology, however, was not so characteristic that it allowed the diagnosis of a basophil leucocyte if no metachromasia could be seen in the cytoplasm.

The appearance of basophil nuclei before and after decolourizing followed by nuclear restaining is shown in Fig. 2 and 3.

Examination of basophils in smears of peripheral blood from patients with urticaria.

Comparison of direct and indirect basophil count in 20 patients with urticaria yielded a good correspondence between the results (table 3). No partially degranulated cells, which

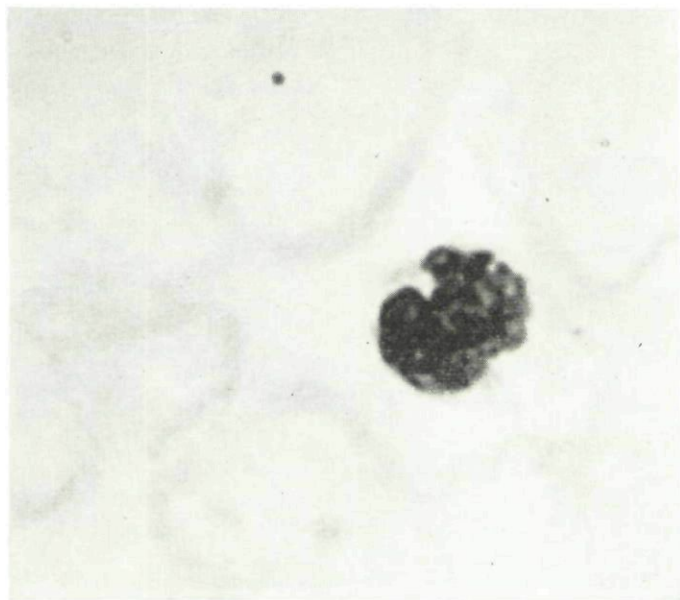


Fig. 3.

The same basophil as in fig. 2, decolourized with hydrochloric acid and then stained in Giemsa. The nucleus is clearly visible. $\times 2600$.

might have been difficult or impossible to observe in the chamber, were found in the smears. Swelling of basophils was not observed.

Incubation experiments.

Effect of hypotonic solutions on basophils.

The results are given in table 4. It can be seen that slight hypotonia of the solution does not influence the basophil number. Only when distilled water constitutes 80 per cent or more of the incubation solution are all basophils destroyed. Increase of the volume of basophil cells and of metachromatic granules is observed when the tonicity of the surrounding solution is less than half of that of blood.

Increase of basophil cell size preceding the disruption can

thus be observed in hypotonic solution and there is reason to look for enlarged cells under conditions where degranulation of basophils can be expected to occur.

TABLE 3
Comparison of direct and indirect basophil counts in urticaria patients.

Patient no.	Direct count	Indirect count
1	1	0
2	3	0
3	0	0
4	2	4
5	1	0
6	0	0
7	0	0
8	5	0
9	6	2
10	0	3
11	0	3
12	0	0
13	4	0
14	3	12
15	0	4
16	0	6
17	0	0
18	0	0
19	7	3
20	0	0

Effect of heparin on basophils.

The results are given in table 5. High concentration of heparin in the fluid (2500 $\mu\text{g/ml}$) yielded zero values of basophils, and 250 $\mu\text{g/ml}$ decreased the basophil count considerably in two cases although the count was unchanged in the others. In this concentration of heparin it is however very difficult to diagnose the basophil. Lower concentrations of heparin do not influence the basophil count.

Since there was reason to suspect that the absence of stained basophils in high concentrations of heparin might be due to a

reaction between added heparin and toluidine blue, which then could not stain the cells, an experiment was performed in which 2500 μg heparin was added to 1 ml blood for 30 minutes in 37° C. Smears were then prepared and stained according to Undritz. It was then found that basophils were present to the same extent in the heparinized blood as in the blood without added heparin. This finding is in accordance with the assumption that the zero values of basophils in high concentrations of heparin are due to disturbed staining and not to a degranulation of the cells.

TABLE 4

In vitro effect of hypotonic solutions on basophils. Counts are made after incubation at 37° C for 30 minutes. The figures represent per cent values and the basophil count in isotonic solution = 100 per cent.

Number of sample	10	20	30	40	50	60	70	80	90
1	76	112	132	100	79	32	13	0	0
2	89	71	66	112	91	78	14	0	0
3	119	123	95	116	131	107	19	0	0
4	79	81	73	61	60	50	5	0	0
5	109	113	102	76	102	76	37	0	0
6	87	107	105	85	75	63	11	0	0
7	93	91	84	89	83	78	11	0	0
8	116	102	83	112	107	102	20	0	0
9	107	81	91	83	71	61	6	0	0
10	102	95	114	102	86	73	21	0	0
Mean	98	98	95	94	89	72	16	0	0

Effect of histamine on basophils.

The results of incubation of basophils in high concentrations of histamine (100 $\mu\text{g}/\text{ml}$) are given in table 6. There was no increase of cell volume. It is evident that histamine has no effect on the basophil count in the dosage used, which is far above the plasma concentrations physiologically present in man.

TABLE 5

In vitro effect of heparin on basophils. From each specimen of cell suspension samples are incubated with and without heparin. Counts are made after incubation at 37° C for 30 minutes. The figures represent per cent values of the number of basophils in the sample without heparin (controls).

Specimen	Basophil counts in per cent of control			
No.	2500 μ g heparin/ml	250 μ g*) heparin/ml	25 μ g heparin/ml	2.5 μ g heparin/ml
1	0	113	76	75
2	0	11	83	104
3	0	64	114	83
4	0	7	121	117
5	0	27	118	95
Mean	0	44	102	95

* Faint metachromasia of the cells made diagnosis difficult.

TABLE 6

In vitro effect of histamine (100 μ g/ml) on basophils. From each specimen of cell suspension samples are incubated with and without histamine. Counts are made after incubation at 37° C for 30 minutes. The figures represent per cent values of the number of basophils in the sample without histamine (control).

Specimen no.	Basophil counts in per cent of control
1	123
2	94
3	108
4	122
5	96
Mean	109

Effect on basophils of serum from patients with urticarial basopenia.

The effect of serum from patients with urticarial basopenia on basophils from healthy persons is seen in table 7.

A slight decrease of basophil count was observed in the controls, i.e., in serum from persons with normal basophil counts. Basophil counts after incubation in urticaria serum are

expressed as per cent of the counts after simultaneous incubation in normal serum. It is evident from the table that serum from urticaria patients with basopenia has no decreasing effect on basophil count as compared with normal serum. No increase of the cell volume of basophils could be observed.

TABLE 7

In vitro effect of serum from patients with urticarial basopenia on basophils from healthy persons. From each specimen of cell suspension samples are incubated with basopenic and normal serum. All samples are incubated at 37° C for 30 minutes. The figures represent per cent values of the number of basophils in the sample incubated with normal serum (control).

Urticaria serum No.	Number of basophils per mm ³ blood of urticaria serum donor	Basophil counts in per cent of control
1	0	122
2	0	81
3	0	72
4	3	93
5	0	83
6	0	103
7	0	107
8	0	91
9	1	106
10	0	119
Mean		98

Cellular changes in the urticaria wheal.

To make sure that basophils are preserved during the technical treatment of the biopsies, dextran-separated and centrifuged leucocytes from persons with normal percentage of basophils were treated in the same way as the skin biopsies. It was then found that basophils were present to the same extent in the sections as in the smears from the dextran-separated leucocyte-suspension. The basophils are easy to identify and there is hardly any risk that they are mistaken for tissue mast cells, which never exhibit metachromatic granulation of such

TABL
Cellular changes in the urticaria wheal. Under clinical remar
 ++ moderate number of wheal

Specimen No.	Sex	Age	Age of wheal	Baso-phils/mm ³	Eosino-phils/mm
1	F	49	3 hours	3	210
2	M	60	6 hours	0	250
3	M	25	15 minutes	30	188
4	F	41	2 hours	8	352
5	M	58	30 minutes	5	119
6	F	42	3 hours	11	56
7	M	35	4 hours	3	75
8	M	49	2 hours	12	113
9	M	34	30 minutes	0	44
10	F	25	20 minutes	0	69
11	F	27	4 hours	2	63
12	F	53	1 hour	2	63
13	F	59	2 hours	6	138
14	F	43	2 hours	6	250
15	F	13	4 hours	15	169

is given the degree of symptoms at biopsy. + means a few wheals,
and +++ numerous wheals.

Clinical remarks and degree of symptoms	Cellular reactions in the wheal
Frequent bouts of urticaria for 2 years. ++	No basophils. Slight degranulation of mast cells.
Symptoms for 2 weeks. ++	No cellular infiltrate.
Dermographism for 5 years. ++	No basophils. Moderate degranulation of mast cells.
Pulmonary sarcoidosis. For 9 years urticaria mainly with upper respiratory infections. +++	Slight, mainly perivascular infiltrate of eosinophils.
Symptoms for 3 months. ++	No basophils. Moderate degranulation of mast cells.
Symptoms for 2 months. +++	No cellular infiltrate.
Symptoms for 3 months. +++	No basophils. Moderate degranulation of mast cells.
Chromium dermatitis. Urticaria for 2 months. ++	Dense, diffuse infiltrate of neutrophils and eosinophils.
Urticaria for 8 months. +	Karyorrhexis of leucocytes.
Repeated bouts of urticaria last year. +	No basophils. Pronounced degranulation of mast cells.
Arthritis and urticaria at present annular for 2 years. ++	No cellular infiltrate.
Pretibial myxoedema. Urticaria for 3 weeks. +	A few basophils. Slight degranulation of mast cells.
Penicillin urticaria for 4 days. +++	Dense, diffuse infiltrate of neutrophils and eosinophils.
Arthritis and urticaria for 3 months. ++	No basophils. Pronounced degranulation of mast cells.
Almost daily urticaria for 3 years. ++	Strong diffuse infiltrate of neutrophils and eosinophils.
	A few perivascular basophils. Mast cells partly degranulated. Slight infiltrate of eosinophils, mainly perivascular.
	No basophils. Very slight degranulation of mast cells.
	A few perivascular neutrophils and eosinophils.
	No basophils. Slight degranulation of mast cells.
	Slight intra- and perivascular accumulation of eosinophils.
	No basophils. Moderate degranulation of mast cells.
	Strong diffuse infiltrate of neutrophils and eosinophils, with considerable karyorrhexis.
	No basophils. Moderate degranulation of mast cells.
	Moderate, mainly intravascular accumulation of eosinophils.
	A few perivascular basophils. Massiv degranulation of mast cells. Dense diffuse infiltrate of eosinophils and neutrophils.
	A few perivascular basophils. Mast cells partly degranulated. Dense diffuse accumulation of eosinophils and neutrophils.
	No basophils. Mast cells only slightly degranulated.
	Very few perivascular eosinophils.

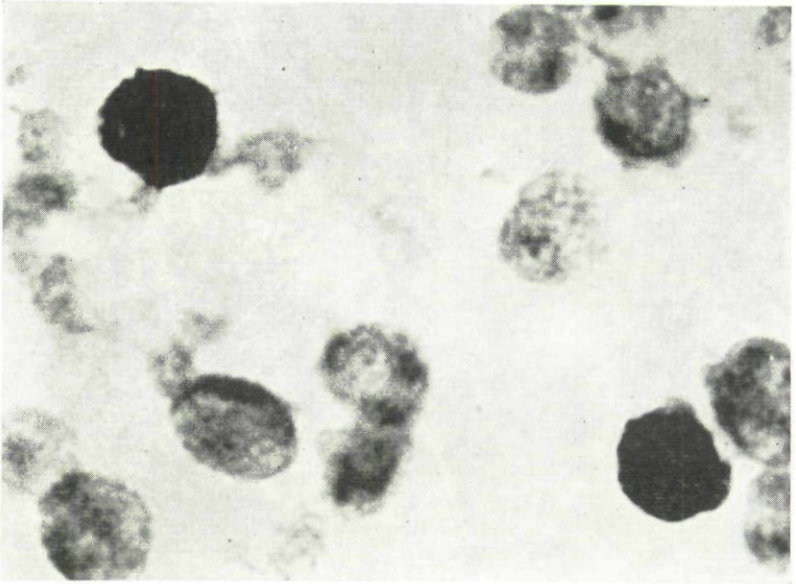


Fig. 4.

Two basophil leucocytes in a leucocyte suspension, technically treated as a skin specimen. $\times 2600$.

density (Fig. 4). In basophils with sparse granulation the segmented nucleus is diagnostic (Fig. 5).

The cellular reactions in the wheals and some clinical data of the patients examined are given in table 8. Edema and vascular dilatation present in different degrees are not specially recorded.

In six cases there was a pronounced cellular infiltrate, in six cases the cellular infiltration was moderate or slight, and in three cases no cellular infiltration could be seen. The abundance of eosinophils was striking in many cases with cellular infiltration.

Only in three patients were basophils present to a small extent in the cellular infiltrate of the wheals. In no case were basophils found in the normal skin.

The mast cells were mostly located around the vessels and could sometimes be found only there. Degranulation of mast

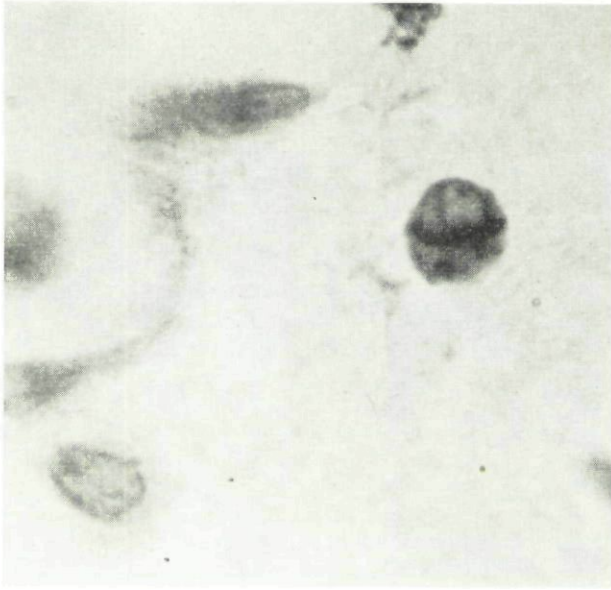


Fig. 5.

Partly degranulated basophil in a skin specimen from patient with urticaria.
 × 2600.

cells of different degrees was observed in all cases. However, it should be mentioned that degranulated mast cells were often found also in sections from apparently normal skin. Further, entirely normal mast cells without any sign of degranulation could be observed in the middle of a pronounced inflammatory infiltrate.

Excretion of neutral 17-ketosteroids and 17-ketosteroidogenic steroids.

The results are given in table 9 and illustrated in Fig. 6 and 7.

The excretion of neutral *17-ketosteroids* was *increased* in two cases and *decreased* in one. Of the two patients who had increased excretion one was a female, aged 61, with a diet regulated diabetes, and a basophil count of 5 cells per mm³,

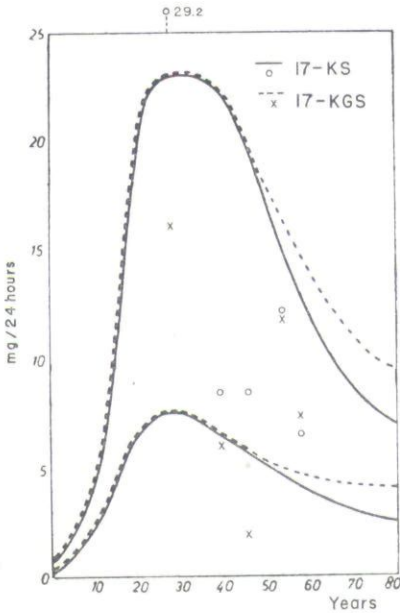


Fig. 6.

Urinary excretion of 17-KS and 17-KGS in five males with urticaria. The lines represent 95% probability normal distribution curves.

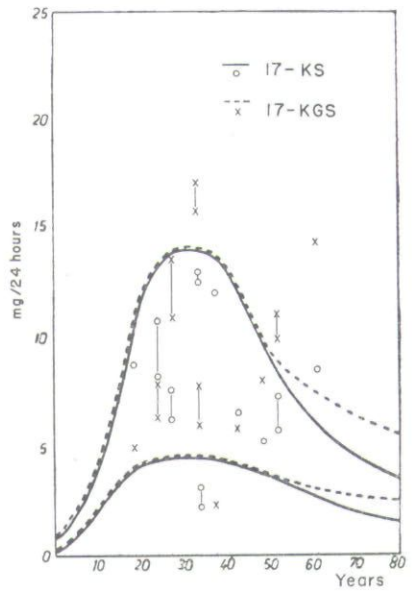


Fig. 7.

Urinary excretion of 17-KS and 17-KGS in ten females with urticaria. The curvelines represent 95% probability normal distribution curves. Vertical lines connect results from determinations during two days in the same person.

and the other a male, aged 28, with 6 basophils per mm^3 . The last-mentioned patient had an increased excretion of neutral 17-ketosteroids even two months after the urticaria symptoms had vanished. However, he did not show any symptoms of endocrine dysfunction. His basophil count two months after the urticaria had ceased was 16 cells/ mm^3 . The patient with decreased excretion of neutral 17-ketosteroids, who had signs of general endocrine hypofunction, was a female aged 33. She developed Addison's disease two years later. Her basophil count was zero.

The excretion of 17-ketosteroidogenic steroids was found to be *increased* in three patients, all females. One of these pat-

TABLE 9

*Excretion of neutral 17-ketosteroids (17-KS) and of 17-ketosteroidogenic steroids (17-KGS).
The degree of symptoms is indicated as in table 8.*

Patient No.	Sex	Age	17-KS mg/24 h.	17-KGS mg/24 h.	Bas./ mm ³	Eos./ mm ³	Degree of symptoms	Remarks
1	M	58	6.7	7.3	22	250	+	Dental apical osteitis. Aggravation by certain kinds of food.
2	F	38	12.3	2.3	0	25	++	
3	F	33	2.3	7.9	0	0	+++	Amenorrhea for 3 years. Dental apical osteitis. Developed Addison dis. after 2 years.
"	"	"	3.2	6.1	0	0		
4	F	52	5.8	11.2	0	121	+++	Pretibial myxoedema after thyroidectomy. Pernicious anemia.
"	"	"	7.4	10.1	0	63		
5	M	40	8.6	6.1	0	100	++	Aggravation by acetylsalicylic acid.
6	F	17	8.9	5.0	0	125		
7	F	42	6.7	6.0	8	25	++	Urinary infection. Joint pains.
8	M	54	12.4	11.9	1	6	++	Myasthenia gravis. Thymectomized. Pneumonia. Dental inflammation.
9	M	46	8.6	1.9	0	50	+++	
10	M	28	29.2	16.2	6	119	+	
"	"	"	30.5	24.3	16	144	—	Two months after end of symptoms.
11	F	61	8.7	14.5	5	81	++	Diet controlled diabetes.
12	F	49	5.3	8.1	0	0	++	Aggravation of infection, certain kinds of food, acetylsalicylic acid.
13	F	27	6.4	11.0	8	63	++	Arthritis. Gammaglobulin 1.83 %.
"	"	"	7.8	13.8	7	19	++	
14	F	34	13.2	15.9	26	150	+	Cholinergic urticaria.
"	"	"	12.8	17.2	37	75	+	
15	F	24	10.9	8.0	1	44	++	
"	"	"	8.3	6.4	8	113	++	

ients, aged 34, had cholinergic urticaria with a basophil count of 37 cells per mm³. The second patient was a female, aged 52, with pretibial myxoedema after thyroidectomy. She had also pernicious anemia. Her basophil count was zero. The third patient was the one with diabetes and an increased excretion

of 17-KS. Her basophil count was 5 per mm^3 . *Decreased* excretion of 17-KGS was found in three patients, two males, aged 40 and 46, and one female, aged 38. Their basophil counts were zero. None of them had symptoms of endocrine disease.

DISCUSSION

The per cent count of basophils in the bone marrow of patients with urticaria was about the same as in patients with psoriasis, who have normal basophil counts in peripheral blood, and the counts in these two conditions also agreed with the basophil count in the normal subjects. Our normal basophil counts do not seem to differ from those given for normal subjects in other materials (*Rohr* 1949). Classification of basophils according to the degree of maturity has been performed (for references see *Rohr* 1949). As a differentiation seems to be very uncertain without special staining procedures, this was not made in the present study.

The finding of a normal number of basophil myeloid cells in bone marrow specimens from patients with urticarial basopenia is of importance. Thus, the marrow basophil counts do not support the assumption of a decreased formation of basophils or of basophil retention in the marrow during urticaria. Instead, the findings agree with the assumption that the basophils are destroyed or degranulated in the circulation or trapped outside the circulation, e.g., in the reacting tissue. The presence of basophils in the bone marrow in spite of the peripheral basopenia may be due to special properties of the basophils which have not yet entered into the general circulation, or to special protecting conditions in the bone marrow. Their entering the circulation seems to be a prerequisite for the disappearance of the cells.

It is known that not only myeloid metachromatic cells but also metachromatic tissue mast cells may be present in the bone marrow (*Riley* 1959). *Moeschlin* (1947) describes the granules of tissue mast cells as fine, in contrast to the coarser granules in basophils. The granules in tissue mast cells are

mainly located in the periphery of the round or oval nucleus, which is visible in contrast to the basophil nucleus generally covered by the coarse and confluent granules.

In only one of the patients with urticaria, were tissue mast cells observed in the bone marrow. Basophils in the peripheral blood was here zero and basophils in the bone marrow 35 per 10,000 myeloid cells. Tissue mast cells in the bone marrow of anemic patients have been described by *Undritz* (1946), *Bremy* (1950), *Fadem* (1951), and *Messerschmitt* (1954). *Johnstone* (1954) considered that the occasional appearance of tissue mast cells in the bone marrow of man had no significance, but *Rohr* (1949) never found tissue mast cells in the normal bone marrow. *Riley* (1959) agreed with *Williams* (1952) that mast cells in the bone marrow had the same significance as elsewhere, being a normal component of the connective tissue. We did not find any explanation of the presence of tissue mast cells in our patient.

According to *Arneth* (1920), it should be possible to diagnose basophil leucocytes merely from the appearance of the nucleus. The study of the nuclear morphology of leucocytes first identified by metachromatic staining with toluidine blue decolourized with hydrochloric acid and then restained with Giemsa, showed that the chromatin of basophils was often more compact than in other leucocytes. Further, the nuclear segments in basophils were often closer to one another than in other granulocytes in smears of the same thickness. However, we did not succeed in recognizing basophils only from their nuclear appearance.

A total degranulation of basophils would make it impossible to identify the cells. If basophils at an assumed degranulation retained a few metachromatic granules, these would make it possible to identify the cells in smears, although the scarceness of granules would preclude the identification of the cells during counting in chamber. We therefore made an examination with respect to partly degranulated cells but obtained no evidence for a subtotal degranulation of basophils in urticaria.

It is well known that tissue mast cell degranulation may be

produced in vitro through antigens or special chemicals, histamine liberators (*Högberg & Uvnäs*, 1957 and 1958; *Uvnäs & Thon* 1959; *Humphrey & Mota* 1959; *Boréus* 1960). Degranulation of mast cells is preceded by swelling of the cells (*Uvnäs & Thon* 1959). If a degranulation of basophils occurs this could also possibly be preceded by swelling which might be easier to observe than a partial degranulation. The capacity of basophils to swell before disruption was tested in hypotonic solutions. It was then found that the basophils tolerated fairly pronounced hypotonia and that swollen cells could be observed in hypotonic solutions which left many basophils undisrupted. Although the mechanism regulating the swelling of basophils in hypotonic solutions may be quite different from that giving rise to swelling of mast cells incubated, e.g., in allergen solution (*Uvnäs & Thon* 1959), there seems to be reason to look for swollen basophils in conditions where degranulation of these cells may be assumed to occur.

The metachromatic granules of tissue mast cells probably contain heparin (*Jorpes* 1946; *Bloom* 1960; *Ringertz* 1960). Release of metachromatic granules has been described in urticaria (*Asboe-Hansen* 1950) and heparin has been reported to influence the basophil count *in vivo* (*Angeli, Tedeschi & Cavazzuti* 1955; *Braunsteiner & Thumb* 1958; *Ardoino* 1958; *Piette & Piette* 1959; *Boseila* 1959). In the course of the experiment on the effect of heparin on basophils in vitro it was found that high concentrations of heparin resulted in zero counts of basophils by the chamber method. This, however, was not due to any degranulation of the cells, as basophils could be demonstrated in smears prepared from blood with high concentration of heparin. It seems probable that the absence of basophil leucocytes at chamber count is due to insufficient staining through binding of available amounts of toluidine blue by the added heparin. In the staining of smears, however, toluidine blue is present in excess and the staining of basophils may therefore be sufficient.

The presence of histamine in tissue mast cells is now firmly established (*Riley* 1959). The important rôle of this amine in

urticaria is evident from the work of *Lewis* (1927). *Nilzén* (1947) demonstrated a decrease of the amine in the reacting skin, and *Adam, Hunter & Kinnear* (1950) found an increase of the urinary excretion of free histamine in some cases of urticaria, which indicates liberation of histamine in the tissue. As injected histamine can produce a change in the basophil number (*Leo* 1956) it seems probable that also endogenously liberated histamine might influence the basophils. Therefore, the effect of histamine on basophils *in vitro* was examined. The absence of any decrease of basophils and also of swelling of the cells in the very high concentrations of histamine, far above biological levels, favours the assumption that the histamine has no direct effect on the basophils.

It could be suspected that serum from patients with urticarial basopenia contained a substance responsible for the disappearance of the basophil cells. The reactions of basophils from healthy persons incubated in serum from patients with urticaria were therefore compared with the basophil reactions in serum from persons with normal levels of basophils. The counts were the same in the serum from urticaria patients as they were in the normal serum. No increase of basophil cell volume was found.

Literature on the histology of the wheal was studied with regard to the hypothesis that basophils could accumulate in the reacting skin tissue. *Unna* (1887) and *Darier* (1923) did not find any cellular reactions in the wheal. Many other authors have reported accumulation of neutrophils, eosinophils or lymphocytes in the whealing tissue (*Vidal* 1880; *Raymond* 1892; *Bäumer* 1896; *Kaposi* 1899; *Gilchrist* 1908; *Hodara* 1913; *Jadassohn & Rothe* 1914; *Török, Lehner & Kenedy* 1922; *Török* 1928; *Berger & Lang* 1931 and 1932; *Jadassohn* 1932; *Kline, Cohen & Rudolph* 1932; *Miescher* 1952; *Prakken & Woerdemann* 1952; *Werner* 1953; *van Ufford* 1959). None of the mentioned authors, however, observed basophil leucocytes in the whealing tissue. As the absence of basophil leucocytes in the wheal might be due to the technique used, fixation and staining in our study were performed with

special reference to the metachromatic substances. Basic lead acetate was used for fixation of mast cells (*Sylvén* 1941; *Asboe-Hansen* 1951; *Schubert & Hamerman* 1956) and toluidine blue, buffered to pH 5, was used for staining (*Montagna & Melaragno* 1953; *Schubert* 1956; *Schubert & Hamerman* 1956). The suitability of our fixation and staining for the demonstration of basophil leucocytes was confirmed in an experiment on separated leucocytes. The basophils were found to be present in the same number in the histologic specimens as in smears directly prepared from the solution and stained in alcoholic toluidine blue according to *Undritz* (1950).

Basophils were found to a limited extent in three out of the 15 wheals studied. The sparse infiltration of basophils in these cases of urticaria does not seem to afford any good explanation for the urticarial basopenia in peripheral blood. It must be considered possible that basophils lose their granules rapidly in the whealing tissue. Such degranulated basophils cannot be demonstrated.

There are some reports on accumulation of basophils in non-hematopoietic tissues. In man, hematogenous mast cells have been reported in the skin in such diseases as scabies, prurigo Hebræ, dermatitis herpetiformis, eczema, miliaria crystallina, erythema exsudativum multiforme, herpes zoster and herpes simplex (*Klausner & Kreibich* 1909 and 1913). No change of the basophil count in the peripheral blood, however, was reported in these conditions.—In rabbits and guinea-pigs injection of protein induces local basophilia (*Ringoen* 1923; *Plimpton* 1940). Protein injections may also induce basophilia in the bone marrow and in the circulating blood (*Winqvist* 1960). In rabbits a pronounced local accumulation of basophils in ovarian and oviduct vessels and around ruptured follicles may be seen at ovulation (*Zachariae, Asboe-Hansen & Boseila* 1958). This local basophilia is combined with basopenia in the circulating blood. The authors considered that "the accumulation of basophils might be a reaction to the local edema, an attempt on the part of the organism to bind, i.e., organize the water to a mucinous substance, a hydrated gel".

From table 5 it appears that the change of basophils in the *peripheral blood* is pronounced, while normal eosinophil counts are observed in the majority of urticaria patients. In the *wheal*, however, the eosinophil is a far more predominant cell than the basophil in those cases where a cellular infiltrate is present. The explanation for this may be that, in contrast to basophils, the eosinophils have special functions in the whealing tissue. Recent data, which suggest that the eosinophils have antihistamine and antiheparin activity (Kovacs 1950; Vercauteren 1953; Archer 1960) support this hypothesis. Another explanation may be that accumulated basophils are more easily degranulated than eosinophils are. It seems possible that degranulation of accumulated basophils combined with histamine liberation may be an initial stage in the wheal formation and a mechanism starting the degranulation and histamine liberation of tissue mast cells. The initial accumulation of fluid, which is by some authors considered to precede the mast cell degranulation (Wegelius & Boseila 1958; Riley 1959), might be produced through histamine liberated from basophils. Graham, Lowry, Wheelwright, Lenz & Parish (1955) considered that basophils "may constitute a mobile source of a limited amount of histamine immediately available wherever needed to tide over any lag period before the arrival of more adequate supplies from a nearby fixed source".

Degranulation of mast cells in the wheal was pronounced in some of the studied cases but undamaged cells could be seen in spite of considerable inflammatory changes. The presence of intact mast cells is not surprising as it is known that considerable histamine release can take place without any change in the mast cell morphology (West 1956). In some cases, considerable degranulation of mast cells was found in biopsies from unaffected skin but previous whealing in these sites could not always be excluded. No quantitative determinations of the number of mast cells or of their degree of degranulation were performed. There are some previous studies on mast cells in whealing conditions. Hodara (1913) and Sezary, Caroli & Horovitz (1952) reported numerous mast cells in urticaria

factitia. *Asboe-Hansen* (1951) found numerous mast cells in the perivascular tissue but few in the intervascular connective tissue of whealing skin in urticaria factitia and in spontaneous urticaria. He also found signs of expulsion of granules from the mast cells in these conditions. *Levi, Meneghini & Rantuccio* (1959) found no difference in mast cell count in normal and whealing skin in two studied cases of urticaria. The many sources of error in the determination of mast cells in the skin have been pointed out by *Asboe-Hansen* (1951).

The hypothesis that basopenia in urticaria is generally due to adrenal hyperfunction is not supported by the determinations of 17-KS and 17-KGS in 15 patients. Only two patients had an increase of 17-KS excretion, and three had an increase of 17-KGS excretion. Furthermore, one patient with increase of 17-KGS excretion had cholinergic urticaria without basopenia. This shows that moderate increase in the adrenal function need not necessarily decrease the basophil count. Decrease of 17-KS excretion was found in one patient who later got Addison's disease. This patient had zero basophils. Decrease of 17-KGS excretion was found in 3 other patients, one female and two males, without any symptoms of endocrine dysfunction. All three had zero basophils. In such cases a non-adrenal mechanism for basopenia must be assumed.

In addition, the fact that basopenia in urticaria is not regularly combined with eosinopenia militates against the hypothesis that adrenal hyperfunction is the main cause of urticarial basopenia, since it is known that ACTH and cortison decrease eosinophils more than basophils (*Code et al.* 1954; *Osada* 1954; *Boseila & Uhrbrand* 1958). In severe urticaria eosinopenia is common, and the study of *Roskam, van Cauwenberge & Lecomte* (1956) showed that hyperexcretion of adrenal steroids occurs in severe urticaria, while mild urticaria shows normal adrenal steroid excretion. It is probable that adrenal hyperfunction is a mechanism inducing basopenia in severe cases of urticaria.

SUMMARY

Investigations were made into possible mechanisms causing urticarial basopenia. The results were as follows:

Examination of the bone marrow cytology in patients with urticarial basopenia shows normal basophil counts.

There is no partial degranulation of basophils in the peripheral blood of urticaria patients. If total degranulation of basophils occurs, it is impossible to identify the cells.

Swelling of basophils precedes disruption of the cells in hypotonic solutions. Basophils do not swell and are not degranulated in heparin or histamine solutions or in serum from patients with urticarial basopenia.

Accumulation of basophils in the wheal is not regular and never pronounced.

Increased excretion of 17-KS and 17-KGS is not generally found in moderate urticaria.

The present findings agree with the assumption that degranulation or destruction of the basophils in the circulating blood or in the wheal is the cause of urticarial basopenia.

REFERENCES

- Adam, H. M., Hunter, R. B. & Kinnear, T. W. G.*: Observations on the urinary excretion of histamine in urticaria. *Quart. J. Exp. Physiol.* 36, 49, 1950.
- Angeli, G., Tedeschi, G. & Cavazzuti, F.*: Sulle variazioni del tasso dei leucociti basofili del sangue periferico dopo somministrazione di eparina. *Minerva med.* 46, 752, 1955.
- Appleby, J., Gibson, S., Norymberski, J. K. & Stubbs, R. D.*: Indirect analysis of corticosteroids. I. The determination of 17-hydroxy-corticosteroids. *Biochem. J.* 60, 453, 1955.
- Archer, R. K.*: Studies with eosinophil leucocytes isolated from the blood of the horse. *Brit. J. Haemat.* 6, 229, 1960.
- Ardoino, A. L.*: Modifications des cellules sanguines après injections de protamines chez le lapin. *Rev. Hem.* 13, 311, 1958.
- Arneth, J.*: Die qualitative Blutlehre. Leipzig, Klinkhardt's Verlag, p. 477, 1920.
- Asboe-Hansen, G.*: A survey of the normal and pathological occurrence of the mucinous substances and mast cells in the dermal connective tissue in man. *Acta dermat.-venereol.* 30, 338, 1950.

- Asboe-Hansen, G.*: Om bindevævet mucinøse substanser. Experimentelle undersøgelser over hyaluronsyrens oprindelse, forekomst og variabilitet i mesenchymale væv, specielt i huden. København, Rosenkilde og Bagger's Forlag, Thesis, Københavns Universitet, 1951.
- Bäumer, E.*: Beiträge zur Histologie der Urticaria simplex und pigmentosa. Arch. Dermatol. u. Syph. 34, 323, 1896.
- Berger, W. & Lang, F. J.*: Zur Histopathologie der idiosynkrasischen Entzündung in der menschlichen Haut. Beitr. path. Anat. 87, 71, 1931.
- Berger, W. & Lang, F. J.*: Ein histopathologischer Beitrag zur Histaminhypothese der allergischen Reaktion. Ztschr. Hyg. 113, 206, 1932.
- Bersani, R. B.*: The blood basophil in urticaria and other dermatoses. Thesis, University of Zürich, 1959.
- Bloom, G.*: Studies on the Cytology, Cytochemistry and Pathology of the Mast Cell. Stockholm, Roos Boktryckeri AB, Thesis, Karolinska Institutet, Stockholm, 1960.
- Boréus, L. O.*: A comparison of mast-cell reactions in the rat, hamster and guinea-pig. Acta physiol. scand. 49, 251, 1960.
- Boseila, A.-W. A.*: Influence of heparin and serotonin on basophil leucocytes of rabbit blood. Experientia 15, 343, 1959.
- Boseila, A.-W. A. & Uhrbrand, H.*: Basophil-eosinophil relationship in human blood. Studies on the effect of corticotrophin (ACTH). Acta endocrinol. 28, 49, 1958.
- Braunsteiner, H. & Thumb, N.*: Quantitative Veränderungen der Blutbasophilen und ihre klinische Bedeutung. Acta hæmat. 20, 339, 1958.
- Bremy, P.*: Die Gewebasmastzellen im menschlichen Knochenmark. Stuttgart, Georg Thieme, 1950.
- Code, C. F. & Mitchell, R. G.*: Histamine, eosinophils and basophils in the blood. J. Physiol. 136, 449, 1957.
- Code, C. F., Mitchell, R. G. & Kennedy, J. C.*: The effect of cortisone on the number of circulating basophils and eosinophils: Is there a relationship between these cells? Proc. Staff Meet. Mayo Clin. 29, 200, 1954.
- Darier, J.*: Précis de Dermatologie. Paris, Masson & Cie, p. 32, 1923.
- Fadem, R. S.*: Tissue mast cells in human bone marrow. Blood 6, 614, 1951.
- Genell, S., Jensen, C. C. & Lindegård, B.*: On the quantitative relationship between some steroid substances excreted in the urine by adult males. Lunds Universitets Årsskr. N. F. 2, vol. 52, pt. 6, 1956.
- Gilchrist, T. C.*: Some experimental observations on the histopathology of urticaria factitia. J. cutan. diseases 26, 122, 1908.
- Graham, H. T., Lowry, O. H., Wheelwright, F., Lenz, M. A. & Parish, Jr., H. H.*: Distribution of histamine among leukocytes and platelets. Blood 10, 467, 1955.
- Hodara, M.*: Untersuchungen über die Histologie der Urticaria factitia (Dermographismus) und der Urticaria chronica, sowie über die Mastzellen bei beiden Affektionen. Dermat. Wchnschr. 57, 971, 1913.

- Högberg, B. & Uvnäs, B.*: The mechanism of the disruption of mast cells produced by compound 48/80. *Acta physiol. scand.* 41, 435, 1957.
- Högberg, B. & Uvnäs, B.*: Inhibitory action of Allicin on degranulation of mast cells produced by compound 48/80, histamine-liberator from ascaris, lecithinase A, and antigen. *Acta physiol. scand.* 44, 157, 1958.
- Humphrey, J. H. & Mota, I.*: The mechanism of anaphylaxis: Specificity of antigen-induced mast cell damage in anaphylaxis in the guinea-pig. *Immunology* 2, 31, 1959.
- Jadassohn, J. & Rothe, L.*: Zur Pathogenese der Urticaria. *Berlin Klin. Wchnschr.* 51, 519, 1914.
- Jadassohn, W.*: Eosinophilie und urticarielle Quaddel. *Arch. Dermat. u. Syph.* 166, 458, 1932.
- Johnstone, J. M.*: The appearance and significance of tissue mast cells in human bone marrow. *J. Clin. Path.* 7, 275, 1954.
- Jorpes, J. E.*: Heparin in the Treatment of Thrombosis. 2nd ed. London, Oxford University Press, 1946.
- Kaposi, M.*: Pathologie und Therapie der Hautkrankheiten, 5th ed., Berlin, Urban & Schwarzenberg, p. 335, 1899.
- Kelemen, E. & Bikich, G.*: Insufficiency of acute response of basophil and eosinophil leucocytes and of blood histamine after the administration of ACTH and cortisone in untreated leukemia. *Acta hæmat.* 15, 202, 1956.
- Klausner, E. & Kreibich, C.*: Über exsudative Mastzellen. *Arch. Dermat. u. Syph.* 96, 235, 1909.
- Klausner, E. & Kreibich, C.*: Über den Mastzellengehalt vesiculöser Hauteflorescensen. *Folia hæmat.* 15, 347, 1913.
- Kline, B. S., Cohen, M. B. & Rudolph, J. A.*: Histologic changes in allergic and nonallergic wheals. *J. Allergy* 3, 531, 1932.
- Kovacs, A.*: Antihistamine effect of eosinophil leukocytes. *Experientia* 6, 349, 1950.
- Leo, P.*: Comportamento dei leucociti basofili circolanti dopo somministrazione di istamina ed istamina + antiistaminici. *Rass. med. sarda* 7, 442, 1956.
- Levi, L., Meneghini, C. L. & Rantuccio, F.*: Activité cholinésterasique: pouvoir histaminolytique et dosage de l'histamine dans le peau de sujets sains et atteint de certaines dermatoses allergiques: rapport histamine - mastocytes. *Acta allergol.* 13, 332, 1959.
- Lewis, T.*: The Blood Vessels of the Human Skin and their Responses, London, Shaw, 1927.
- Meneghini, C. L. & Levi, L.*: Istamina cutanea et mastociti in alcune dermatosi pruriginose. *Minerva dermat.* 33, 247, 1958.
- Meneghini, C. L. & Levi, L.*: Istaminemia e mastociti circolanti in alcune dermatosi allergiche. *Giorn. It. Dermat.* 100, 449, 1959.
- Messerschmitt, J.*: Fréquence des Mastzellen dans les frottis de moelle osseuse chez l'homme. *Rev. hemat.* 9, 189, 1954.
- Miescher, G.*: Histologie der allergischen Reaktionen. *Proc. first int. Congr. Allergy, Zürich 1951, Basel/ New York, S. Karger, p. 137, 1952.*

- Moeschlin, S.*: Die Milzpunktion. Basel, Schwabe & Co., 1947.
- Montagna, W. & Melaragno, H. P.*: Histology and cytochemistry of human skin. III. Polymorphism and chromatropy of mast cells. *J. Invest. Dermat.* 20, 257, 1953.
- Moore, J. E. & James, G. W.*: A simple direct method for absolute basophil leucocyte count. *Proc. Soc. Exper. Biol. Med.* 82, 601, 1953.
- Nilzén, Å.*: Studies in histamine (H-substance) with special reference to the conditions obtaining in urticaria and related skin-changes. *Acta dermat.-venereol.* 27, suppl. 17, 1947.
- Noah, J. W. & Brand, A.*: The effect of corticosteroid administration upon blood histamine content. *J. Allergy* 28, 405, 1957.
- Osada, Y.*: Parellel changes in the numbers of circulating eosinophils and basophils induced by ACTH administration. *Bull. Inst. Pub. Health* 4, 12, 1954.
- Piette, M. & Piette, C.*: Études sur les granulocytes basophiles du sang. II. — Injection intraveineuse d'heparine chez le lapin. *Ann. pharm. fr.* 17, 344, 1959.
- Plimpton, Jr., N. C.*: Basophil leucocytes and myelocytes after local injection of ventriculin. *Anat. Rec.* 76, 475, 1940.
- Prakken, J. R. & Woerdeman, M. J.*: Mast cells in diseases of the skin; their relation to tissue eosinophilia. *Dermatologica* 105, 116, 1952.
- Raymond, P.*: Note sur l'anatomie pathologique de l'urticaire et de la dermographie. *Gazette des Hôpitaux civils et militaires* 65, 1393, 1892.
- Riley, J. F.*: The Mast Cells. Edinburgh and London, E. & S. Livingstone Ltd., p. 20, 1959.
- Ringertz, N. R.*: Acid Polysaccharides of Mast Cell Tumours, Uppsala, Almqvist & Wiksells Boktryckeri AB, Thesis, Karolinska Instiutet, Stockholm, 1960.
- Ringoen, A.*: The mast leucocytes in the adult guinea-pig under experimental conditions. *Am. J. Anat.* 31, 319, 1923.
- Rohr, K.*: Das menschliche Knochenmark. Stuttgart, Georg Thieme, p. 71, 1949.
- Rorsman, H.*: Basophil leucocytes and urticaria. *Acta dermat.-venereol.* 37, 121, 1957.
- Rorsman, H. & Rosengren, E.*: Basophil leucocytes and blood histamine in urticaria. *Acta dermat.-venereol.* 38, 377, 1958.
- Roskam, J., van Cauwenberge, H. & Lecomte, J.*: Excrétion urinaire des corticostéroïdes au cours de certaines réactions et maladies allergiques. *Int. Arch. Allergy* 9, 102, 1956.
- Schubert, J. C. F.*: Differenzierungsmethode metachromatischer Zellen nach ihren Säuregrad. *Experientia* 12, 346, 1956.
- Schubert, M. & Hamerman, D.*: Metachromasia; chemical theory and histochemical use. *J. Histochem.* 4, 159, 1956.
- Sezary, Caroli & Horowitz* quoted in *de Granciansky, P. & Boule, S.*: Atlas de Dermatologie, Paris, Libraire Maloine, 1952.

- Sylvén, B.*: Über das Vorkommen von hochmolekulären Esterschwefelsäuren im Granulationsgewebe und bei der Epithelregeneration. *Acta chir. scand.* 87, suppl. 66, 1941.
- Tatai, K. & Osada, Y.*: The adrenocortical stimulating effect of adrenochrome derivatives as administered to healthy young males. *J. med. Progr., Jap.* 32, 441, 1957.
- Thonnard-Neumann, E.*: Basophile Leukozyten und Steroidhormone. *Medizinische*, 1087, 1958.
- Thonnard-Neumann, E.*: The influence of hormones on the basophilic leucocytes. *Acta hæmat.* 25, 261, 1961.
- Torii, T. et al.*: *Jap. J. Hematology* 161, 94, 1953; quoted by *Osada*.
- Török, L.*, in *Jadassohn, J.*: *Handbuch der Haut- und Geschlechtskrankheiten*. Berlin, Julius Springer, 1928, vol. 6, pt. 2, p. 153.
- Török, L., Lehner, E. & Kenedy, D.*: Untersuchungen über die Pathogenese und pathologische Anatomie der Urticaria. *Arch. Dermat. u. Syph.* 139, 141, 1922.
- van Ufford, W. J. Q.*: Urticaria und Quincke's oedema, in *Jamar, J. M.*: *International Textbook of Allergy*, Copenhagen, Munksgaard, p. 413, 1959.
- Undritz, E.*: Die nicht zur Blutkörperchenbildung gehörenden Zellen intravitaler Knochenmarkspunkate nebst Auszählungsschema für Myelogramme. *Schweiz. med. Wschr.* 76, 333, 1946.
- Undritz, E.*, in *Hämatologischer Tafeln Sandoz*, Basel, Sandoz AG, p. 23, 1950.
- Unna, P. G.*: Beiträge zur Anatomie und Pathogenese der Urticaria simplex und pigmentosa. *Dermat. Studien* 3, 7, 1887; appeared also as *Monatsh. prakt. Dermat.* 6, suppl. 1, 1887.
- Uvnäs, B. & Thon, I.-L.*: Isolation of "biologically intact" mast cells. *Exptl. Cell Research* 18, 512, 1959.
- Vercauteren, R.*: The properties of isolated granules from blood eosinophils. *Enzymologica* 16, 1, 1953.
- Vidal, E.*: De l'urticaire. *Annales de dermatol. et de syphiligr.*, p. 408, 1880.
- Wegelius, O. & Boseila, A.-W. A.*: Effect of water and physiological saline solution on mast cells in the peritoneal fluid of the rat and hamster. *Exptl. Cell Research* 11, 437, 1956.
- Werner, M.*: Die allergischen Testreaktionen. *Int. Arch. Allergy* 4, 425, 1953.
- West, G. B.*: XX. International Physiological Congress, Brussels, p. 964, 1956.
- Williams, G. T.*: Tissue mast cells in human bone marrow. *Am. J. Clin. Path.* 22, 1039, 1952.
- Winqvist, G.*: Experimental production of basophil granulocytes in the guinea-pig. *Exptl. Cell Research* 19, 7, 1960.
- Zachariae, F., Asboe-Hansen, G. & Boseila, A.-W. A.*: Studies on the mechanism of ovulation. Migration of basophil leucocytes from blood to genital organs at ovulation in the rabbit. *Acta endocrinol.* 28, 457, 1958.

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