

Automated Measurement of Human Basophil Degranulation

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Basophils disappear after challenge with specific antigen. A human basophil degranulation test has been carried out on a slide using an enriched cell suspension. On each slide, the same volume of cell suspension was deposited in wells with antigen diluted in buffer or with buffer only (control). The basophil count was made on an equal number of randomly distributed microscopic fields either by eye (optical) or by image analyser (automatic).

In 35 subjects, the correlation coefficient between the numbers of non-degranulated basophils counted by eye and by image-analyser on control wells was found to be $r = 0.91$, $P < 10^{-8}$.

In 12 patients (6 with hydatidosis and 6 with schistosomiasis), the percentage of degranulation with three antigen dilutions was measured. The correlation between the results obtained by eye and by image-analyser reached 0.76 ($P < 10^{-7}$).

The authors now use the automated measurement of human basophil degranulation routinely.

Key words: anaphylaxis; degranulation; human basophils.

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IgE-bearing basophils lose their metachromatic granulations about 10 to 15 min after challenge with optimal dilutions of specific antigens (9) and then apparently disappear. This fact has not been widely applied routinely for diagnostic purposes on native blood in man because the number of the cells is small (30 to 50/ μ l). However, after partial enrichment, a human basophil degranulation test (HBDT) becomes valid in the diagnosis of foods (10), mites (3), bee and wasp sting allergies (8), pollinosis (2) and in helminthiasis (1, 5).

The aim of this work was to estimate the accuracy of an automated measurement of degranulation with an image analyser compared with optical measurement.

absence of antigen in order to assess concordance between optical and automated counting of non-degranulated basophils.

In 12 patients, six with a hepatic hydatid cyst recently proved by surgery (5), and six with schistosomiasis demonstrated by the presence of viable eggs in urine, stools or at rectal biopsy (1), we carried out an HBDT with specific antigens to assess concordance between the two counting techniques in degranulation. In these cases, the IgE-mediated mechanism of degranulation had been previously shown: specific degranulation by hydatid liquid was totally inhibited by EDTA (6) while no degranulation appeared in control subjects with these two antigens (1, 5).

PATIENTS AND METHODS

Patients

Two groups of subjects were studied: In 35 subjects basophil counts were carried out in

Basophil degranulation test

All reagents and slides were kindly supplied by D. Chenebaux, Institut Pasteur Production (I.P.P.), 28, rue du Docteur Roux, 75015 Paris.

The technique previously reported (7) has

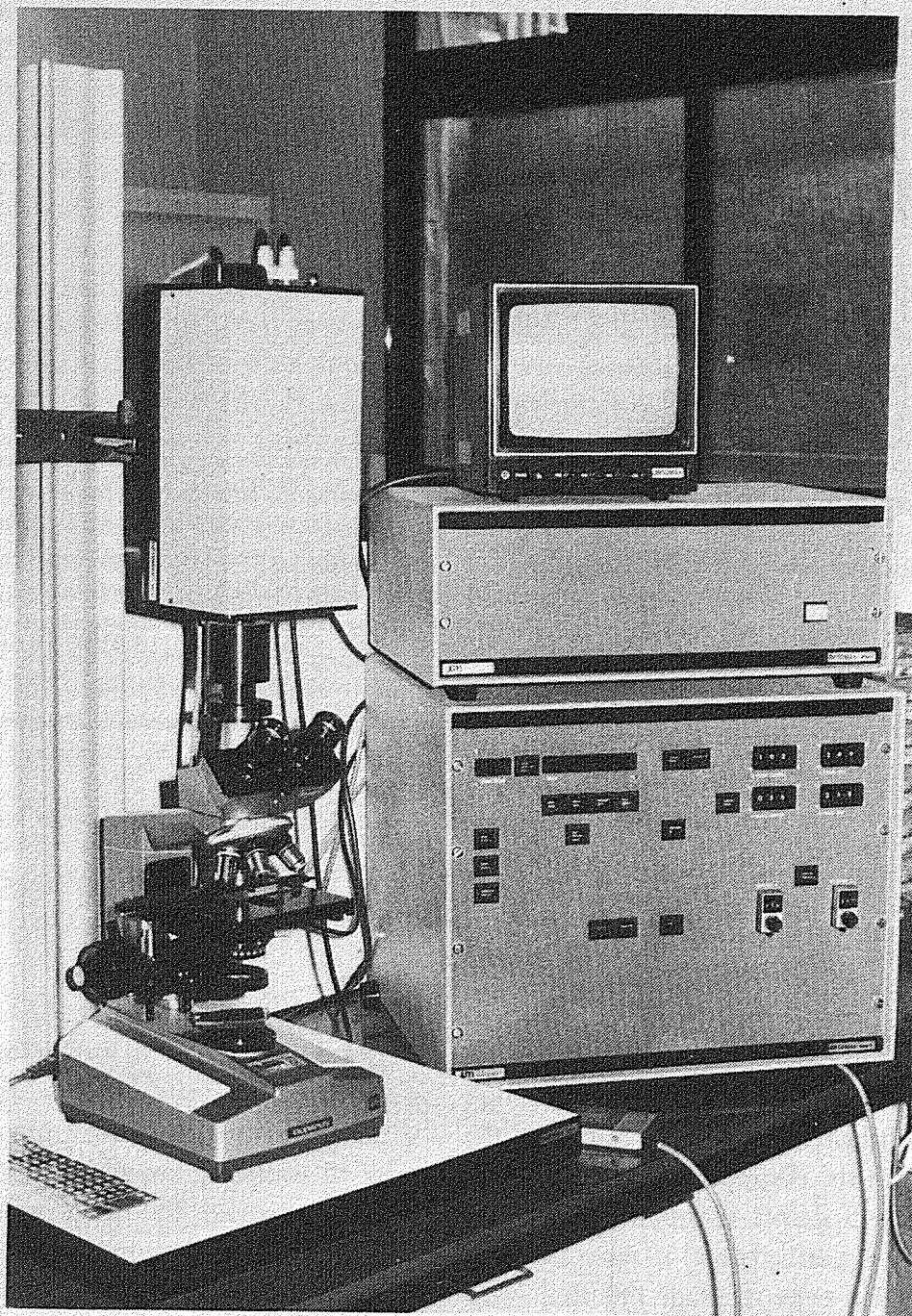


Fig. 1. The microscope, camera, video and computer.

been simplified. In brief, 10 ml blood collected by venipuncture into heparin (Venoject®) were centrifuged (15 min, 150 g) to separate platelet rich plasma, which was then discarded. The buffy coat was collected and mixed with 5 ml of Hepes buffer and then gently deposited on 5 ml of gradient density (1,080 g/ml) containing sodium metrizoate 9.05% (Nyegaard, Oslo) and Ficoll 7.6%. (Pharmacia). After centrifugation (30 min, 400 g at the interface, 20°C), the ring containing basophils monocytes and lymphocytes was collected and washed once in 15 ml Hepes buffer (10 min, 150 g). The supernatant

buffer down to about 0.5 ml from the bottom of the tube was discarded. After stirring, 20 µl of this enriched cell suspension and 10 µl of each antigen dilution (or 10 µl of buffer as control) were deposited in wells on a slide for immunofluorescence with eight wells. On each slide there were two wells for control and two wells for each dilution of antigen. The slide was then maintained for 15 min at 37°C in a Petri dish containing a piece of wet cotton wool, before quick drying with hot air and then simultaneous staining and fixing with a solution containing toluidine blue. Finally, the slide was mounted.

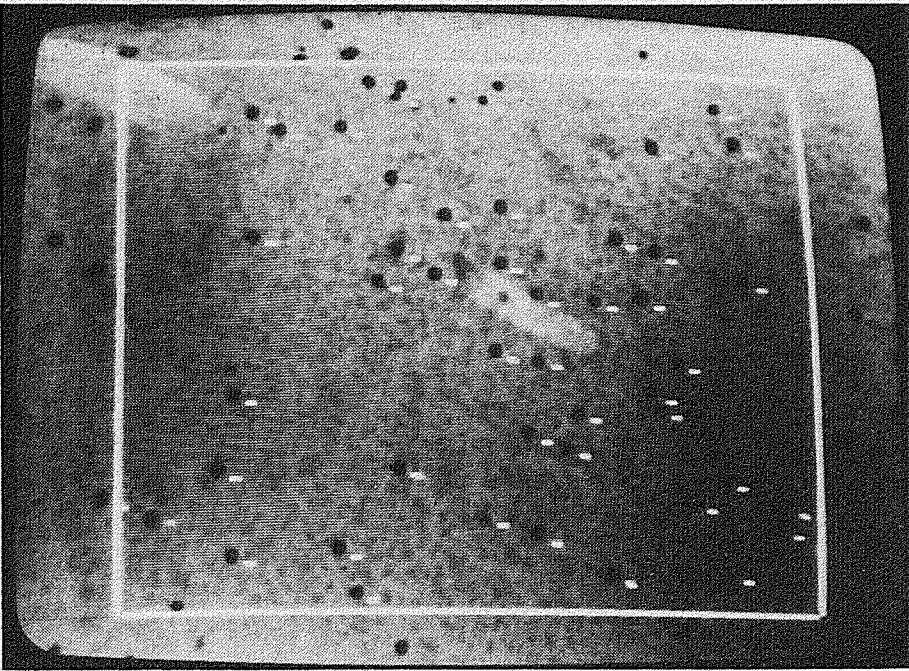


Fig. 2. The video control. Each basophil appears as a dark spot marked by a "flag".

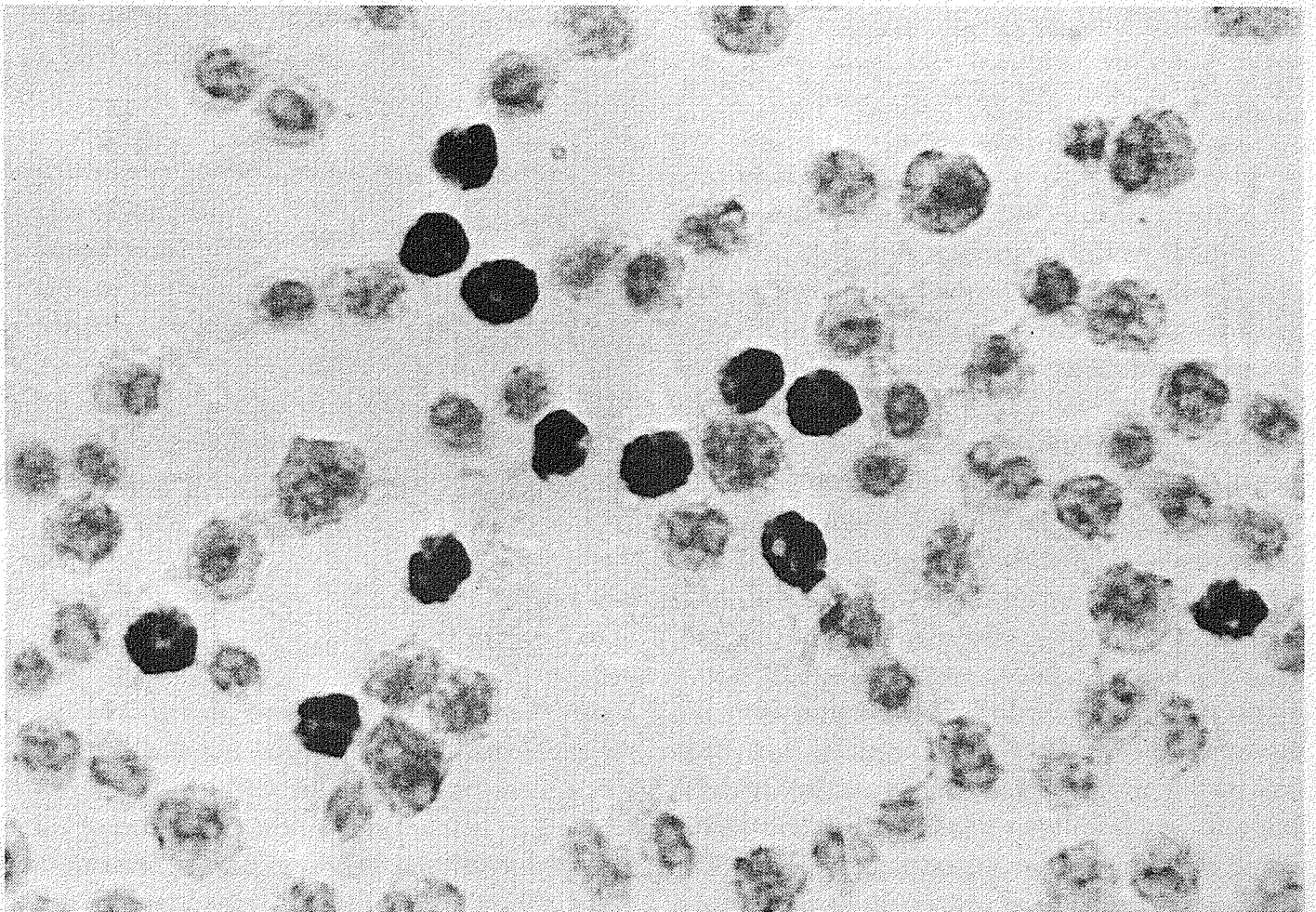


Fig. 3. Non-degranulated basophils at magnification 10×40 .

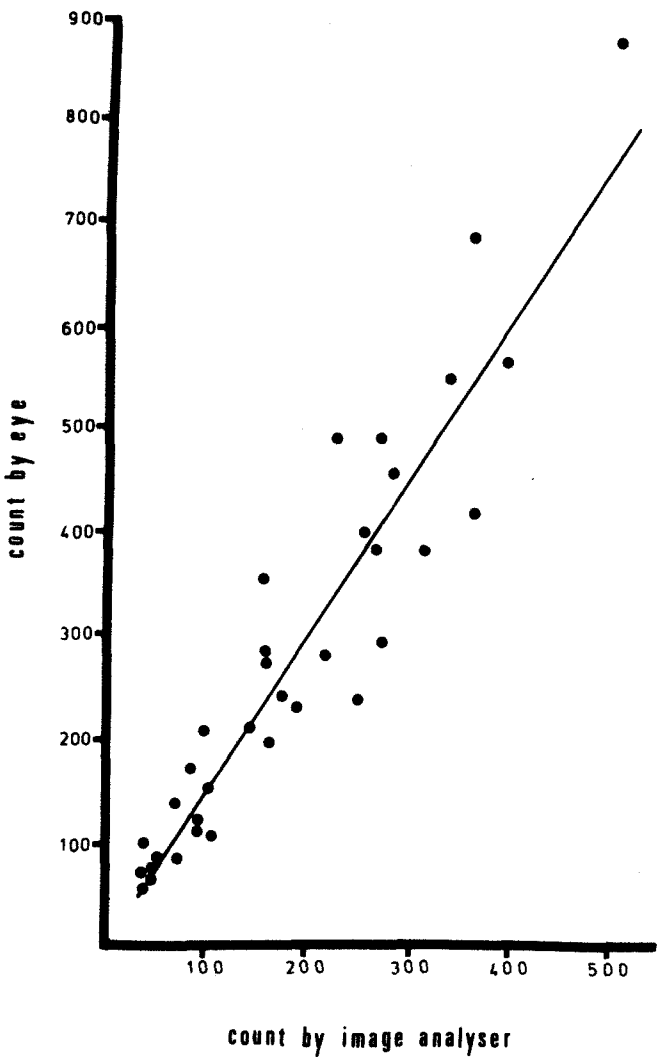


Fig. 4. Correlation between number of non-degranulated basophils counted in 36 subjects using optical measurement and image analyser ($r = 0.91$, $P < 10^{-8}$).

Antigens

Antigens were freeze-dried *Schistosoma mansoni* (I.P.P.) diluted at 100, 10 and 1.0 µg/ml in buffer or hydatid liquid drawn from a hydatid cyst in a patient (5) and used pure and diluted at 1:10 and 1:100. Antigen dilutions were kept in aliquots at -80°C until use.

Basophil count

As previously reported (2, 7), basophils from 10 randomly distributed microscopic fields in each well were counted (20 fields for control and 20 fields for each antigen dilution). Degranulation was expressed as the percentage of basophils which apparently disappeared in the presence of antigen.

Table 1
Percentages of degranulation with three antigen dilutions measured by two counting methods. Patients No. 1-6 had hydatidosis and patients No. 7-12 schistosomiasis

Patient	Optical	Image analyser
1	92	91
	82	80
	60	71
2	73	71
	66	70
	47	48
3	74	60
	59	56
	55	56
4	65	83
	84	80
	10	47
5	80	57
	93	57
	94	80
6	46	38
	48	13
	22	24
7	53	58
	67	60
	0	5
8	88	70
	69	73
	40	49
9	54	67
	37	63
	0	35
10	98	88
	95	76
	72	70
11	91	63
	77	68
	23	10
12	92	55
	85	68
	33	0

Visual counting was done with a Zeiss microscope (Standard 14) at 10 × 40 magnification, and automated counting with an image analyser

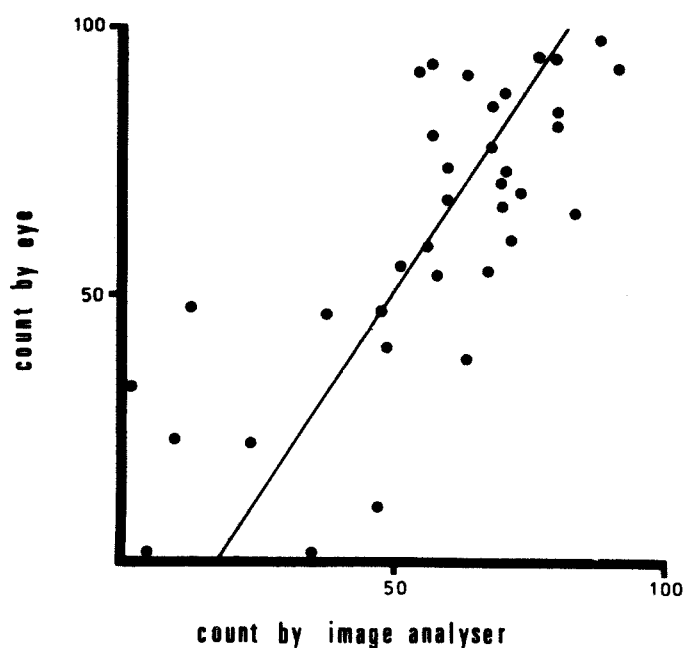


Fig. 5. Correlation between percentage of degranulation using optical measurement and image analyser ($r = 0.76$, $P < 10^{-7}$) in 12 subjects (three dilutions of antigen for each subject).

(Optomax® micromasurement – Systèmes Analytiques, 4 rue Sainte Famille, 78000 Versailles).

The normal basic image analyser system consists of the following units (Fig. 1):

- central processor
- scanner
- power supply
- video monitor
- seat of leads
- microscope interface

An Olympus microscope (BHB) was used at 10×25 magnification. A green light filter absorbed the red purple colour of the basophils, which became darker on the video control (Fig. 2).

RESULTS

Generally, it was possible to count three to 10 non-degranulated basophils from each field in control wells at 10×40 magnification (Fig. 3). The number of non-degranulated basophils counted in 35 subjects (20 microscopic fields for each) is shown in Fig. 4.

The number was lower with the image ana-

lyser than with the eye, because the field area was smaller. However, there was high degree of correlation ($r = 0.91$, $P < 10^{-8}$) between these two counts.

Table 1 and Fig. 5 shows the percentage of degranulation observed in 12 patients with three dilutions of antigen. The correlation between these 36 measurements reached $r = 0.76$ ($P < 10^{-7}$).

DISCUSSION

The HBDT for diagnostic purposes in allergies and helminthiasis has been reported. In previous works on mite allergies (3) and pollinosis (2), using antigens provided by B. David (Institut Pasteur, Unité, d'Immuno-allergie) we have observed degranulation curves very similar to curves obtained with the same antigens by B. David with fluorimetric histamine release (personal communication). The accuracy of the results depends on the number of basophils counted in the control wells (2, 3, 4, 7, 8).

Thus a partial enrichment is necessary (7, 8). With our technique it was possible to count by eye, more than 100 basophils in the control wells. However, counting basophils in 10 fields per well on an eight-well slide (two wells for control and two wells for each antigen dilution) was time-consuming, i.e. 10–15 min per slide.

As the basophils appeared darker than other cells, especially under green light, it was possible to make them stand out, because of their grey level of colour, on an image analyser using the camera video system.

The correlation between counts by eye and counts by image analyser was highly significant for non-degranulated basophils ($r = 0.91$). The correlation between the percentage of degranulation obtained in 36 measurements (3 antigen dilutions for 12 patients) was less satisfactory ($r = 0.76$). Nevertheless, with the image analyser, degranulation was always higher than 35% for at least one antigen dilution in all patients, which corroborated the diagnosis for all of them (Table 1). (2).

The main disadvantage with the image analyser is the artefacts; dust particles on the slide or cover-slip have the same grey-colour level as

basophils and get falsely counted as basophils by the computer.

With the image analyser it takes less than 5 min to count a slide even if several microscopic fields are examined. Moreover it is easier for the technician who only has to work with the video control (Fig. 2). Automatic slide adjustment on the microscope platform would give more accuracy and allow many more fields to be counted in the same time. Nevertheless, we now use the image analyser for routine HBDT.

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