was followed by disappearance of the neck node. 2 months later, tumor recurred in the left thigh muscles. A 4th graft was followed by temporary regression, then enlargement of the thigh mass. The mouse was autopsied when found dying. Large blood containing spaces were present in some areas of tumor, the characteristic appearance of tumors undergoing postgraft regression. Death appeared due to a combination of the effects of a moderate tumor load, tumor necrosis (see below), and renal disease. This mouse survived in good clinical condition for 11 months after the diagnosis of multinodal tumor, more than one third of the life span of the longest survivors of this hybrid (personal observation). 1 Tx and 2 intact mice which presented originally with a single or 2 enlarged peripheral lymph nodes showed complete node regression after a single graft, and remained tumor free for 26, 21, and 25 months respectively. 1 died from a nonlymphomatous tumor and 2 from lymphoma.

2 serious life shortening complications of grafting were observed, 'tumor necrosis syndrome', and hemorrhage. Usually within 1-4 weeks of grafting, a previously healthy mouse rapidly weakened and died within a few h. At autopsy dark red or black foci of varying size were present in the tumor. Microscopically tumor necrosis resulted in large blood-filled spaces (up to 6 mm in diameter) within the tumor. Pathologic evidence for this syndrome was present in 14 of 18 mice in the Tx and in 7 of 12 mice in the control group. The 2nd complication was that of hemorrhage, usually into necrotic tumor. In 1 such case ending in sudden death, a spleen observed to be lymphomatous at laparotomy was converted by the 12th day after a 2nd graft into a 2.0-g sac of blood with only a few islands of cells.

In these experiments, prolongation of survival and regression of tumor was greater following treatment of small tumors than following treatment of large tumors. The longest survivals were obtained with peripheral nodal tumors, probably for this reason. Nevertheless, with serial grafts, some animals survived and appeared healthy for a large proportion of the normal life span of this strain, as a result of only partial regression or absence of progression of a large or multifocal tumor.

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Chemotaxis is not a special case of haptotaxis[1]

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Summary: Serum peptides containing classical anaphylatoxin (CAT) produce marked chemotactic orientation of human neutrophil granulocytes without modifying cell attachment to the substratum. Furthermore gradients of adhesion produced with gammaglobulins fail to induce morphological orientation of neutrophils. The results suggest that chemotaxis is not a special case of haptotaxis.

Various theories have been developed to explain orientation of cells responding to chemotactic stimuli. One such hypothesis implies that the direction of moving metazoan cells is determined by a gradient of adhesion[2] (haptotaxis[3]). Carter[3] produced gradients of adhesion by depositing palladium on cellulose acetate and reported movement along a gradient of increasing substrate adhesion. He suggested that chemotactic mediators might in one way or another form such gradients of adhesion, which direct moving cells by the relative strengths of their peripheral contacts, and that chemotaxis is therefore just a special case of haptotaxis. Such an inference is necessarily based on the reasonable but unproved supposition that chemotactic mediators specifically increase the strength of adhesion between moving cells and the substratum. The following experiments were performed in order to clarify this point.

The results of such experiments are only conclusive if the following 2 requirements are fullfilled: a) the cells studied must be capable of responding to chemotactic mediators and b) the chemotactic agents used must lack chemokinetic

Relation between neutrophil adhesion and chemotaxis

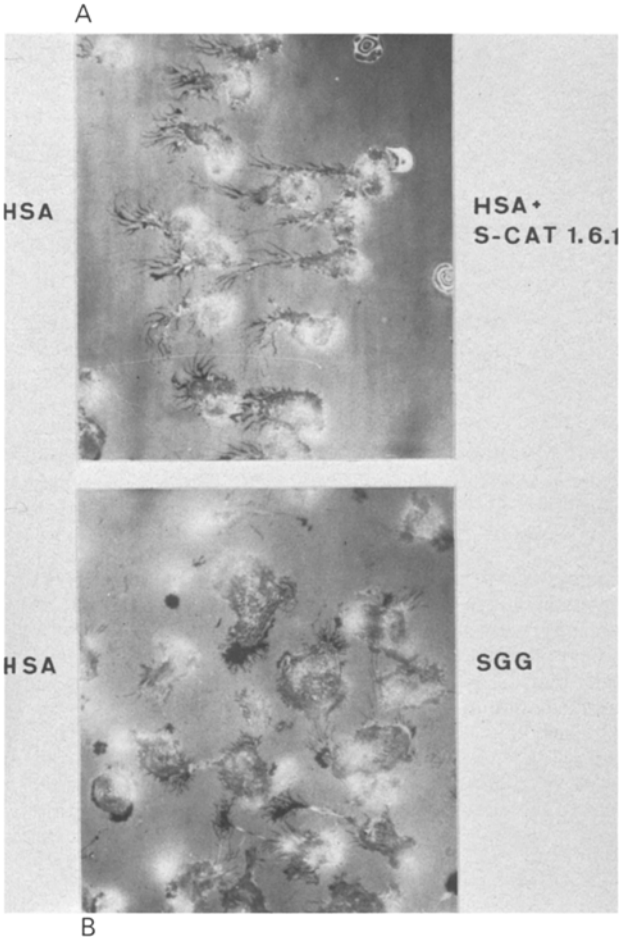
Test material:* Gey's solution containing	% Neutrophils sticking (± SD)	% Neutrophils migrated (± SD) incubation time 1 h	3 h
No addition	54.6 ± 3.7	0.13 ± 0.08	1.9 ± 0.9
HSA (20 mg/ml)	2.8 ± 0.4	0.18 ± 0.04	2.3 ± 0.8
HSA (20 mg/ml) + S-CAT 1.6.1 (4.6 µg/ml)	3.2 ± 1.3	25.2 ± 4.6	50.6 ± 9
HSA (20 mg/ml) + S-CAT 1.6.1 (1.53 µg/ml)	4.9 ± 1.8	29.7 ± 2.0	40.4 ± 4.9
HSA (20 mg/ml) + S-CAT 1.6.1 (0.92 µg/ml)	2.8 ± 1.2	27.8 ± 5.8	48 ± 11.9
HSA (20 mg/ml) + S-CAT 1.6.1 (0.46 µg/ml)	2.3 ± 1.0	21.8 ± 7.1	40.9 ± 10.4
HSA (20 mg/ml) + S-CAT 1.6.1 (0.05 µg/ml)	4.8 ± 0.2	0.58 ± 0.42	5.9 ± 0.6
Standard γ-globulin (20 mg/ml)	65.3 ± 3.6	0.13 ± 0.08	8.4 ± 2

* Neutrophil adhesion was tested using the respective test material as medium for the cells. In contrast, locomotion was tested with cells suspended in 2% HSA in Gey's solution, the respective test material being only present in the lower compartment of the chamber.

activity under the test conditions used, because chemokinetic effects may be mediated by changes in cell adhesion[3-5]. Purified preparations of serum-derived peptides containing classical anaphylatoxin (CAT) and neutrophil granulocytes, respectively fullfill these requirements[6].

Neutrophil granulocytes were obtained from human peripheral blood[7], whereas chemotactic peptide preparations containing classical anaphylatoxin (S-CAT 1.6.1.)[8] were prepared from swine blood as previously described. Human serum albumin (HSA) and standard preparations of human gamma globulin (SGG)[9] were obtained from the Swiss Red Cross Transfusion Service, Bern, and dissolved in Gey's solution at pH 7.2. Neutrophil locomotion was assessed by means of migration chambers with a 2 filter system[7], attachment to glass or cellulose acetate-coated surfaces by means of morphological evaluation of the slides[5] and by reflection-contrast microscopy[10].

We have shown that peptide preparations containing classical anaphylatoxin (CAT) have chemotactic but no chemokinetic properties, when tested in Gey's solution containing 2% HSA[6]. The results presented in the table demonstrate that chemotactic S-CAT 1.6.1. tested over a wide concentration range does not influence the proportion of neutrophils attached to glass in presence of 2% HSA in Gey's solution. In contrast, the standard gamma globulin or Gey's solution alone considerably increased the proportion of neutrophils attached to the slide. This functional test is not always a reliable measure for the adhesive forces between substratum and neutrophils[11], nor does it reflect variations in the pattern of interaction[12]. The pattern of adhesion of individual cells as studied by reflection-contrast microscopy can provide more relevant information[12]. Addition of S-CAT 1.6.1. (no concentration gradient) to neutrophils in Gey's solution containing 2% HSA did not induce any changes in the reflection-contrast pattern (not shown). In contrast, neutrophils in Gey's solution alone or in Gey's solution containing 2% SGG exhibited patterns indicating increased adhesion to the substratum[12] as assessed in the absence of a gradient.



The effect of S-CAT 1.6.1. or SGG on neutrophil orientation was assessed. Neutrophils in Gey's solution were deposited on a coverslip and placed over the bridge of the gradient chamber[13]. The well to the left was filled with Gey's solution containing 2% HSA, the well to the right either with Gey's solution containing 2% HSA and S-CAT 1.6.1. (4.6 µg/ml) or with 5% SGG standard gamma globulin. Neutrophils showed significant orientation in a gradient of S-CAT 1.6.1. (A), but not in a gradient of SGG (B). Neutrophils deposited in Gey's solution alone show black areas representing zones of close contact preferentially at the rear end and grey zones representing zones of intermediate distance contacts towards the front of the cell. Under these conditions reflection-contrast microscopy is particularly suitable to demonstrate orientation.

Direct observation of moving cells in a gradient of S-CAT 1.6.1. or SGG was performed in the chambers described by Zigmond[13]. The gradient of adhesion produced by SGG could be visualized across the bridge by means of reflection-contrast microscopy. The neutrophils located towards the well containing SGG in Gey's solution showed gradually more adhesion than the cells on the other side of the bridge towards the well containing 2% HSA in Gey's solution. In contrast, S-CAT 1.6.1. produced no such visible gradient of adhesion across the bridge. But gradients of S-CAT 1.6.1. (concentration in the well: 0.46 - 4.6 µg/ml Gey's solution containing 2% HSA) produced marked orientation, whereas gradients of SGG (1-50 mg/ml Gey's solution) had no detectable directional effect. This can be particularly well demonstrated with neutrophils that had become attached in Gey's solution (figure). Neutrophils in Gey's solution alone show areas of strong and others of weak adhesion[12]. When these cells move at random or if they become orientated in response to S-CAT 1.6.1. in Gey's solution containing 2% HSA, they exhibit strong adhesion at the rear end of the cell and weak adhesion in the front part (figure). This is in contrast to Carter's hypothesis[3] which implies that adhesion of chemotactically responding cells is stronger in the front as compared to the rear end of the cell. In summary, the findings suggest that chemotaxis is not a special case of haptotaxis.

We see no contradiction between our results and reports showing that chemotactic factors such as formyl-methionyl-leucyl-phenylalanine[14] or casein[15] increase neutrophil adhesiveness under some test conditions and that neutrophils respond to substratum-bound proteins[16]. These agents can also exert marked chemokinetic activity. In view of the close relationship between kinesis and cell adhesion[4, 5, 12], it is extremely difficult to interpret the results of experiments with factors exhibiting chemotactic as well as marked chemokinetic activity. They cannot provide a direct answer to the question whether chemotaxis is a special case of haptotaxis.

The question remains, however, whether haptotaxis is a special case of chemotaxis. Gradients of adhesion can be formed with SGG. The results showed that neutrophils accumulate in response to gradients of SGG in the filter assay particularly at 3 h incubation but exhibit no orientation in the visual assay. The stimulating effect of gradients of SGG was dose-dependent (5-50 mg/ml) and consistently lower than with CAT 1.6.1. (table). These findings may be related to the observations reported by Harris[17] who showed that gradients of adhesion on solid substrata lead to accumulation of cells moving at random, probably as a result of biased kinesis. As orientation is absent, it is unsatisfactory to call this process (hapto)taxis[18]. Thus the

question whether haptotaxis is a special case of chemotaxis cannot as yet be answered. But the results show that chemotaxis is not a special case of haptotaxis. They also indicate that it is premature to conclude that any increased cell accumulation as measured in the filter assay is the result of chemotaxis.

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Changes in beta-2 adrenergic receptor sensitivity with maturation of erythroid progenitor cells[1]

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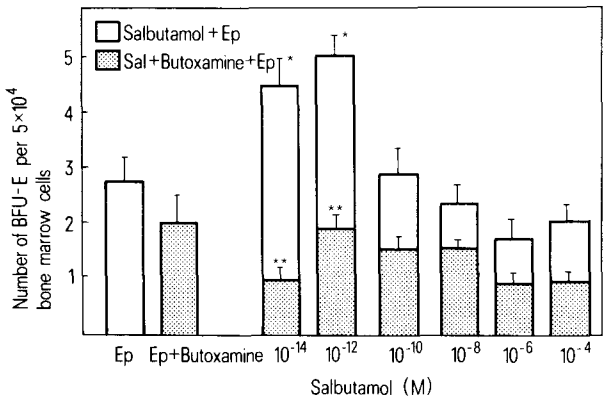
Summary. Erythroid burst forming units (BFU-E) were much more sensitive to the beta-2 selective adrenergic drug, salbutamol, than erythroid colony forming units (CFU-E) in an in vitro study of erythroid progenitor cells.

2 broad classes of erythroid progenitor cells have been described[2,3]. The 1st class, designated as CFU-E (colony forming unit erythroid), forms small erythrocytic colonies consisting of 8–32 cells after 2 days in culture and is considered to be a late stage of progenitor cell development[4]. The 2nd class, BFU-E (burst forming unit erythroid), is recognized by its ability to form large, macroscopic clusters of colonies termed 'bursts' due to their unique morphology. Burst forming units (BFU-E) are thought to be an earlier stage of erythroid progenitor cell development than CFU-E[3,4].

Pluripotent stem cell colonies (CFU-S) and the late stage erythroid progenitor cell colonies (CFU-E) are known to be sensitive to certain drugs and hormones that stimulate the adenylate cyclase-cyclic AMP system such as beta-adrenergic drugs[5–7], growth hormones[8], thyroid hormones[9] and hormones that are independent of cyclic AMP such as steroids[10–12]. Very few studies[13,14] have investigated the responsiveness of the BFU-E compartment to hormones or drugs. Since the BFU-E compartment is intermediate between CFU-S and CFU-E, it might be expected to also be sensitive to those agents which affect CFU-S and CFU-E. Experiments described here further characterize the effects of beta-adrenergic agents on erythroid progenitor cell proliferation by comparing their effects on BFU-E and CFU-E.

Methods. The method of McLeod et al.[15] was modified as described previously[6] for the culture of BFU-E. Briefly, a cell suspension containing 500,000 cells in 0.1 ml of culture medium was dispersed in 0.9 ml NCTC-109 medium containing 20% fetal calf serum, 10% beef embryo extract, 10% L-asparagine, 0.5 units erythropoietin, 10% bovine citrated plasma and the drug to be tested. 0.1 ml of this mixture was pipetted into wells of sterile microtiter plates. The mixture was then allowed to clot and microtiter plates were incubated in sterile 100-mm petri dishes in a humidified atmosphere of 95% air and 5% CO_2 for 10 days at 37 °C. The contents of each well were placed on a microscope slide, blotted gently and fixed with 5% gluteraldehyde. BFU-E colonies were scored if they contained 1000 or more benzidine-positive cells. The Dunnett's multiple range test[16] was employed for statistical analyses.

Results and discussion. The numbers of BFU-E were significantly ($p < 0.05$) increased in the presence of salbutamol, a selective beta-2 agonist. The greatest enhancement was seen at 10^{-12} M salbutamol. In the presence of butoxamine (10^{-8} M), a selective beta-2 antagonist, the salbutamol effect was blocked. In an earlier study[6] we found that erythropoietin-dependent CFU-E formation was enhanced by salbutamol with a peak effect at 10^{-8} M. Butoxamine



Effect of salbutamol, erythropoietin and butoxamine on erythroid burst colony (BFU-E) formation in rabbit bone marrow. Open bars represent the mean ± SE of 5 separate experiments while the stippled bars represent n = 3. * Significantly different from control with Ep alone ($p < 0.05$) using the Dunnett's multiple range test. ** Significantly different ($p < 0.05$) from salbutamol + Ep at same concentration.