Results. The EBP-FCA caused accumulation of lymphocytes and large lymphoid cells in the paracortex and in the medullary cords which was more marked than after injecting NBP-FCA or FCA alone. The development and amount of granulomatous tissue in the hilar area was more prominent in the EBP-FCA and NBP-FCA groups. In the EBP-FCA group, the enlargement of the paracortex was especially prominent; the traffic of lymphocytes through the high endothelium venules was very intensive, and a high proportion of cells of the plasmocytic series were present in the medullary cords which almost compressed the sinuses. Autoradiographic estimates of cellular proliferative activity in the nodes showed that number of proliferating cells significantly increased in both experimental groups. In the control group, L.I. ranged from 1.2% to 1.5%, in the NBP-FCA group from 5.5% to 6.6% and in the EBP-FCA group it was approximately 11%; in the FCA group L.I. was 5.8% (table).

Discussion. Previous work[3] has shown that peak LN wt increment involves a growth of the lymphoid cell population mainly within the first 4 days. Following day 4, the growth of non-specific granuloma prevails. For this reason we concentrated our observations in the present work on the early stages of disease induction. According to previous findings[3], LN growth after NBP-FCA was significantly slower than after EBP-FCA in the same time interval. Present results revealed that EBP-FCA resulted in a more intense accumulation of lymphoid cells in the paracortex and a greater development of granuloma than NBP-FCA or FCA alone. Maturation of plasma cells was also accelerated after EBP-FCA. NBP-FCA, as compared with FCA alone, increased somewhat the growth of the lymphoid cell population and the medullary cords. The significance of the latter must, however, be interpreted with care, since compression from the FCA developing lipogranuloma substantially changes the circulation and architecture of the LN. The latter might be expressed more clearly with EBP and NBP without the addition of FCA. Eliciting EAE without FCA is, however, difficult, if at all possible. Autoradiographic data indicate that the nodal growth in both experimental groups involved proliferation of cells in situ. Previous report[3] showed that growth of the cell population involves also migration of lymphocytes into the LN. Following NBP-FCA injection, both processes are therefore apparently less intense and approximate values for administration of FCA alone. The differences in LN growth and proliferation observed after administration of EBP-FCA and NBP-FCA show that non-specific stimulation of LN by basic protein without encephalitogenic determinant (ED) is relatively small. The presence of ED in the basic protein molecule (EBP-FCA) markedly influenced the local reaction of the LN draining the site of injection in terms of increasing migration of cells into the nodes and proliferation of cells in situ.

Relation between stimulus intensity and neutrophil chemotactic response[1]

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Summary. The effect of chemotactic peptides which lack chemokinetic activity has been investigated. The neutrophil response is proportional to the logarithm of the stimulus intensity, or alternatively a power function with an exponent of 0.3. Equal responses are obtained for equal ratios between the peptide concentration in the lower compartment and the threshold concentration. The significance of Weber-Fechner's law in leucocyte chemotaxis is discussed.

The direction of actively moving leucocytes can be determined by chemical substances in the environment. Since Leber's studies in 1888[3], it is generally believed that this process, called chemotaxis, is instrumental in leucocyte accumulation at inflammatory sites. The relationship between the intensity of the chemotactic signal and the direction finding of leucocytes as expressed in directional locomotion is still largely unknown. The interpretation of earlier data[4–6] was complicated by the fact that the test material influenced the speed of the cells, as well as their direction of locomotion. Thus it had chemokinetic as well as chemotactic activity. It was therefore necessary to evaluate the relationship between stimulus intensity and directionality by means of purified cytotaxin preparations exhibiting chemotactic activity only. Partially purified peptide preparations containing classical anaphylatoxin (S-CAT 1.5.1)[7,8] were prepared from dextran-activated swine serum for such experiments. This preparation contained 4% of chemotactically active peptides[9]. Random and directional locomotion of human peripheral blood neutrophils was assessed with a modified filter technique[10], which provides for stable gradients (unpublished observations). The behaviour of responding cells (random vs directional locomotion) was also determined by direct observation. The results obtained with these 2 techniques were in agreement. Human serum albumin (HSA) had to be present in the test system to permit efficient movement of cells and thereby the expression of chemotaxis in form of directional locomotion[11].

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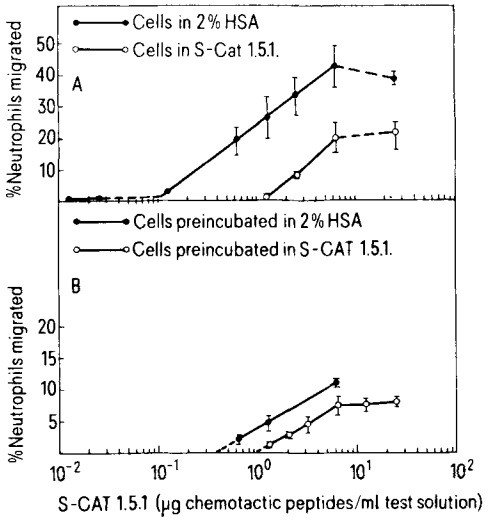
We found that S-CAT 1.5.1. is suitable for the analysis of the chemotactic response. S-CAT 1.5.1. had no measurable activity in absence of human serum albumin. If HSA was present in the medium, a positive gradient between the 2 compartments resulted in attraction, a negative gradient in chemotactic trapping of neutrophils, but S-CAT 1.5.1. did not influence random locomotion measured in absence of a gradient (table). This finding was consistent over a wide concentration range of chemotactic peptides (1.27 µg to 0.00127 µg/ml). Thus S-CAT 1.5.1. determines the direction of the cells *without* affecting the rate of random locomotion, i.e. the rate of random turning and/or the speed of neutrophils. Consequently the increase in the number of cells which have moved through the filter reflects the degree of direction finding by this cell population.

CAT 1.5.1. induces chemotaxis without affecting the rate of random locomotion

Test solution* (lower compartment)	Cell suspending medium* (upper compartment)	Percent neutrophils migrated**
2% HSA	2% HSA	0.38 ± 0.02
CAT 1.5.1. in 2% HSA	CAT 1.5.1. in 2% HSA	0.36 ± 0.07
CAT 1.5.1. in 2% HSA	2% HSA	21.8 ± 2.9
2% HSA	CAT 1.5.1. in 2% HSA	< 0.007

* The basic medium consisted of 2% human serum albumin (HSA) in Gey's solution pH 7.2. CAT 1.5.1. (final concentration 1.27 µg chemotactic peptides/ml 2% HSA in Gey's solution) was added to either the test solution (positive gradient), the cell suspension (negative gradient) or to both compartments (no gradient).
** Values represent the percentage of neutrophils which have migrated through the entire thickness (140 µm) of the filter ± SD[6].



Relation between neutrophil response and test concentration of S-CAT 1.5.1. *A* Locomotion (percent neutrophils migrated through the entire thickness [140 µm] of the filter) of neutrophils suspended in 2% human serum albumin in Gey's solution with or without S-CAT 1.5.1. (0.635 µg chemotactic peptides/ml) towards test solutions containing increasing concentrations of S-CAT 1.5.1. *B* Response of neutrophils which have been preincubated in either 2% HSA or 2% HSA containing S-CAT 1.5.1. (0.635 µg chemotactic peptides/ml) for 30 min at 37 °C. The cells were then washed extensively and resuspended in 2% HSA in Gey's solution.

The increase in the number of neutrophils which have moved through the entire thickness of the filter is proportional to the logarithm of the concentration of S-CAT 1.5.1. in the test compartment (figure, A). Thus the relative increment in external energy corresponds to a constant increment in the response as expressed by cell accumulation. These findings are compatible with our earlier hypothesis[4] that chemotaxis as expressed by directional locomotion is determined by the law of Weber-Fechner. Alternatively we have to consider that the response may be determined by Stevens' power law[12]. It is in fact difficult to distinguish a semilogarithmic relationship (law of Weber-Fechner) between stimulus intensity and magnitude of response from a double-logarithmic power function with an exponent near 0.3 (0.2–0.4), because they may have an almost identical course over several log units[9]. An exponent near 0.3 has been found for human vision and hearing, while other sensory functions have different exponents for power functions relating subjective magnitude to stimulus intensity[12]. Such a relationship provides for a compressor function to match the stimulus from outside to the needs of the cells. In the present experiments, the range of the exponential part of the response is, however, quite small as compared to sensory functions of macroorganisms[12]. The response of normal neutrophils suspended in 2% HSA reaches its plateau at a dose which is up to 50 times above the threshold concentration.

The neutrophils can also respond chemotactically when S-CAT 1.5.1. is present in both compartments of the chamber, provided there is a concentration gradient. The response is again proportional to the logarithm of the attractant concentration in the lower compartment of the chamber until a plateau is reached. The slope of the regression line is not significantly different from that obtained with neutrophils suspended in 2% HSA only (figure, A). Addition of S-CAT 1.5.1. to the cell suspending medium, however, affects other features of the neutrophil response. The threshold is raised above the S-CAT 1.5.1. concentration in the cell-suspending medium (upper compartment), indicating that the cells have responded to positive concentration gradients rather than to the absolute test concentration. The 2 curves take a parallel course (figure, A), suggesting a general relationship. The increase in the neutrophil response is proportional to the ratio between the attractant concentration in the test solution (lower compartment) and the absolute threshold concentration calculated from the regression line

$$\frac{[\text{lower compartment}]}{[\text{absolute threshold}]}.$$

Equal ratios produce equal responses of a particular cell population, even if the threshold is shifted to a different level by the addition of S-CAT 1.5.1. to the cell suspending medium. This is compatible with Weber's principle which says that in order to produce a series of just noticeable differences we must make a fixed relative increase in the stimulus. The addition of S-CAT 1.5.1. also lowers the plateau of the response, indicating that the capacity for a directional response to a positive gradient is reduced. Consequently the linear range in the semi-logarithmic plot of stimulus intensity versus response magnitude is narrowed. This may, however, be more apparent than real, since these cells are in turn in a position to respond to a negative gradient by being trapped (table). The

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slope of the regression lines varied from experiment to experiment which may reflect differences in the average speed of these cell populations.

It has been reported that preincubation of neutrophils in a chemotactic medium renders neutrophils unresponsive to further chemotactic stimulation, even if the cells have been washed extensively. This phenomenon has been called 'deactivation'[13]. We find that neutrophils, which have been exposed to S-CAT 1.5.1. and washed, show normal random locomotion. They behave as if S-CAT 1.5.1. were still present in the cell compartment. The threshold for a chemotactic response of such cells is shifted to a higher concentration of S-CAT 1.5.1. and approximates the cytotaxin concentration used for preincubating the cells. The plateau of the response is lowered (figure, B). This may be considered as a tachyphylactic state in leucocyte chemotaxis. It may reflect a failure to readapt efficiently to an environment with low stimulus intensity. Adaptation, e.g. in visual perception[14] or in chemotaxis of slime moulds[15], is a quite complex phenomenon. Its role in leucocyte chemotaxis remains to be further evaluated.

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The influence of long term estrogen treatment on plasma prolactin levels induced by ether anesthesia in ovariectomized rats[1]

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Summary. 2 methods of continuous estrogen delivery, polyestradiol phosphate injection and implantation of Silastic capsules of estradiol-17 β, in ovariectomized rats induced increases in plasma prolactin in the afternoon (15.00–17.00) beginning at 1 week and continuing for 4–8 weeks. In addition these methods of estrogen treatment potentiated the ether-induced increase in plasma prolactin in the morning (9.00–11.00) beginning on week 2 and continuing for 3–8 weeks. These results indicate that estrogen activates the mechanisms that cause an afternoon surge in prolactin before potentiating a morning elevation induced by ether anesthesia.

Repeated injections of large amounts of estrogen for several days increases the prolactin content in the rat pituitary[2,3], as well as in the serum[4–8]. Estrogen may promote the relase of prolactin into blood by affecting hypothalamic neural mechanisms[9,10]. In contrast to the chronic stimulatory effects of estrogen, several types of stressors induce acute elevations in prolactin secretion. The most widely investigated response is that to ether inhalation. Ether has been shown to increase plasma prolactin in ovariectomized rats[11] and the response is exaggerated in the ovariectomized animal treated with estrogen for 1–3 weeks[12]. The purpose of the present study was to elucidate the effects of long-term estrogen treatment on the ether induced rise of prolactin in the morning when the levels are low, and in the afternoon when the levels are elevated.

Materials and methods. 30 mature, female Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, Michigan) weighing 200–250 g were randomly divided into 6 groups. After 7 days of acclimation to lighting conditions (lights on from 6.00 to 20.00 h) all the rats were bilaterally ovariectomized. 1 group was injected with 0.5 mg polyestradiol phosphate (PEP; 1.0 mg Estradurin®, Ayerst Laboratories) s.c., and 4 groups received s.c. Silastic implants containing 12.5 or 25 mg crystalline estradiol-17 β at the time of ovariectomy. The implants were similar to those used by Legan et al.[14]. 4 sizes of Silastic tubing were utilized: Dow Corning No. 602–265 1.57 mm i.d., 2.41 mm o.d. 14 and 24 mm in length and Dow Corning No. 602–285 1.57 mm i.d., 3.18 mm o.d. also 14 and 24 mm in length. A 2 mm wooden plug was inserted into 1 end of the Silastic tubing and crystalline estradiol-17 β was packed into the open end of the tubing. Another 2 mm plug was inserted into the open end, and both ends were coated with Silastic Medical Adhesive Silicone Type A (No. 890 Dow Corning). The implants were incubated for 30 min in distilled water at 20 °C and wiped with ethanol prior to insertion into a s.c. pocket in the nape of the neck. Vaginal smears were obtained daily to monitor the effects of the estrogen treatments.

Beginning 1 week after ovariectomy and estrogen treatment and continuing once weekly for 8 weeks, a 2 ml blood sample was obtained from the orbital venous plexus using heparinized capillary tubes after 5 min of continuous ether anesthesia in the morning (9.00–11.00 a.m.) and again in the afternoon (15.00–17.00 p.m.). Animals were anesthesized by an initial exposure to ether vapor in a large container, followed by maintenance with a nose cone. The samples were centrifuged and the plasma was collected and stored at –20 °C until assayed.

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