metabolic parameters to sympathomimetics is diminished[9-14]. In accordance with these results, the old mice were unable to mobilize as much liver glycogen as needed to meet the glucose demand and maintain their blood glucose level within a normal range. It appears that not enough substrate can be made available to enable the animals to increase their total metabolism. This, however, means that if more energy is spent for the still considerable increase of motor activity produced by methamphetamine in old mice[2], energy expenditure has to be cut down elsewhere. It seems that in this special case thermogenesis is reduced and no longer compensates for heat loss. This view is supported by the observation that the animals cannot maintain their body temperature when they are kept at an ambient temperature (e.g. 25 °C) which is below their point of thermoneutrality, but that no decline of body temperature occurs if the same experiment is carried out at a room temperature of 34 °C at which heat loss becomes very small.

The finding that in old mice plasma non-esterified fatty acids rise even higher than in juvenile mice, seems to be difficult to reconcile with our hypothesis and with reports that the lipolytic response to sympathomimetics decreases with age[9, 12-14]. One explanation for this phenomenon, which has a parallel in cold-stressed mice[8], may be that plasma levels are only the resultant from the rates of lipolysis and uptake of fatty acids by the various organs and that the oxidation of fatty acids in the citric acid cycle is dependent on the simultaneous degradation of an adequate amount of carbohydrates, which are required to provide the oxaloacetate necessary for the entry into the citric acid cycle of the two carbon fragments of fatty acids[15]. Therefore, utilization of fatty acids is necessarily also impeded when carbohydrates are lacking.

Summary. In contrast to juvenile mice, old mice treated with methamphetamine are unable to adequately mobilize their carbohydrate reserves. They cannot increase their overall metabolism and become hypothermic, while juvenile mice react with increased calorigenesis and hyperthermia.

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Chemotaxis of Rabbit Macrophages in vitro: Inhibition by Drugs

The effect of several drugs on the chemotactic migration of rabbit granulocytes in vitro has been reported in a previous paper[1]. The present work deals with the effect of 15 agents on the chemotaxis of rabbit macrophages.

Materials and methods. Compounds were obtained from the following sources: hydrocortisone succinate as Solu-Cortef from Upjohn; dexamethasone and phenylbutazone from Ciba-Geigy; indomethacin, sodium salicylate and iodoacetic acid from Merck; naproxen from Syntex; vincristine from Lilly; phytohaemagglutinin P from Difco; concanavalin A from Calbiochem; cytochalasin B (phomin) from Sandoz; chloroquine as Resochin from Bayer; dipotassium ethylenediaminetetracetate (EDTA) from Fluka. The cytotoxicity of these compounds for rabbit peritoneal macrophages as judged microscopically by morphological integrity of the cell is included in the Table; it may also be seen that all concentrations tested (except one) were below toxic limits.

The Boyden chamber technique for the in vitro assessment of chemotaxis of mononuclear cells was used[2, 3]. Macrophages were derived from the peritoneal exudates of rabbits injected 4 days earlier with 50 ml of Nujol (Plough Inc., Memphis/Tenn., USA). The average composition of these exudates consisted of approximately 90% macrophages and 10% neutrophils and included a few small lymphocytes. The cells were suspended in modified Gey's solution (10 mg glucose/ml and 2% human serum albumin (HSA; Central Laboratory, Swiss Red Cross, Berne)) and standardized to 4×10^6 cells per chamber. They were mixed with the drug under study immediately before being transferred into the upper compartment of the Boyden chamber. As the incubation in the chamber lasted 5 h as compared with the 2.5 h previously used for neutrophils, preincubation of macrophages with the substance was omitted. The lower compartment contained a solution of 1% casein (according to Hammarsten, obtained from Merck AG, Darmstadt, GFR) in isotonic saline which is an excellent cytotaxin for macrophages concerning potency and reproducibility. Each drug was tested in triplicate chambers and in at least 3 experiments where exudates from different rabbits were used. Positive and negative controls were included in all tests. Normal positive chemotaxis towards casein was determined with cells to which Gey's solution instead of drug was added. For the negative controls, casein was replaced by Gey's solution containing 2% HSA. The latter controls were truly negative, as no or occasionally only one or two cells crossed the entire thickness of the filter. The following point should be stressed: only macrophages having reached the lower surface of the membrane filter were counted. The same batch of micropore filters (Sartorius Membranfilter GmbH, Göttingen, GFR) with a pore size of 12 µm was used throughout these experiments. Macrophages require a large pore size to migrate through a membrane filter about 140 µm thick[3]. The polycarbonate filters with a pore size of 5 µm (Nucleopore) used by

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Inhibition of rabbit macrophage chemotaxis in the BOYDEN chamber

Substances tested	Minimal toxic level for macrophages (μg/ml)	Concentration used for chemotaxis test (μg/ml) [a]	Chemotaxis test in vitro: No. of migrated cells; treated cells as % of controls (Mean ± SE)	No. of tests (*n*)
Antiinflammatory drugs				
Hydrocortisone succinate	>1,000	1,000 — 100	91.7 ± 7.3 (−) [b]	7
Dexamethasone	100	1	68.4 ± 14.9 (?)	5
Indomethacin	10	1	89.5 ± 9.2 (−)	6
Phenylbutazone	1,000	100 — 1	58.3 ± 4.6 (+)	12
Naproxen	1,000	100	95.4 ± 10.7 (−)	5
Sodium salicylate	>1,000	1,000	38.0 ± 8.2 (+)	9
Inhibitors of cell division				
Colchicine	>1,000	100 — 1	21.2 ± 3.0 (+)	9
Vincristine	1,000	100 — 1	20.4 ± 7.1 (+)	5
Mitogenic agents				
Phytohaemagglutinin P	100	50 — 25	30.3 ± 7.1 (+)	6
Concanavalin A	100	30 — 3	10.4 ± 3.7 (+)	9
Miscellaneous agents				
Iodoacetic acid	30	10	42.5 ± 9.2 (+)	4
Cytochalasin B	>10	10 — 1	3.3 ± 2.4 (+)	10
Chloroquine	10	10	51.1 ± 7.1 (?)	7
Dipotassium EDTA	>1,000	1,000	35.9 ± 7.6 (+)	7
DMSO + Tween 80 (μl/ml)		1 + 0.2	25.3 ± 7.3 (+)	3

[a] Gey's solution was used as diluent. Substances insoluble in water were dissolved as follows: 1 mg dexamethasone or indomethacin in 0.1 ml DMSO (dimethylsulfoxide) plus 0.02 ml Tween 80; 1 mg phenylbutazone or naproxen in 0.1 ml 5% sodium bicarbonate; 1 mg cytochalasin B in 1.0 ml DMSO. Final pH was always adjusted to 7.2. [b] (−), no inhibition; (+), inhibition; and (?), doubtful inhibition. Each test included 1 to 3 concentrations of each substance, each dilution always being assayed in triplicate. The average number of control cells (± SD) having migrated through the entire thickness of the filter in the 25 tests included in the Table was 91.2 ± 52.7 cells per high power field. The numbers of migrated cells incubated with substance are expressed as percentage of the controls. The mean percentage from *n* experiments using the drug concentration indicated is shown for each compound with the standard error of the mean. Tests performed with other concentrations are not presented here.

others[4] possess a thickness of only 10 μm[5]. The double filter technique[6] was omitted, because mononuclear cells do not tend to fall off as easily as polymorphonuclear ones.

Results. The results are summarized in the Table. The antiinflammatory drugs phenylbutazone and sodium salicylate clearly inhibited chemotaxis of macrophages in vitro. However, hydrocortisone succinate, indomethacin and naproxen did not appreciably affect cell migration. The interference of 1 μg/ml of dexamethasone with chemotaxis of mononuclear phagocytes seemed only marginal, but at 10 μg/ml inhibition identical to that produced by the solvent dimethylsulfoxide (DMSO) plus Tween 80 alone was found as indicated in the Table. When the solvent was diluted 10 times or when 1 μl/ml DMSO alone was tested, no inhibitory effect could be observed, thus indicating Tween 80 to be responsible for the impaired macrophage migration. The inhibitors of cell division colchicine and vincristine, as well as the 2 mitogens phytohaemagglutinin P and concanavalin A, all exerted a pronounced inhibitory effect on the chemotactic migration of mononuclear cells. Among the miscellaneous group iodoacetic acid markedly, and cytochalasin B completely, prevented macrophage migration. A concentration of 10 μg/ml of chloroquine was toxic for macrophages, although 50% of the cells still succeeded in crossing the filter. Those remaining on top of the filter were damaged to varying extents. Dipotassium EDTA at 1 mg/ml was very effective in preventing cellular migration but, at the lower concentration of 100 μg/ml, this activity was lost (78.5% ± 12.7; 4 test).

Concentrations other than those indicated in the Table have also been used. Tenfold higher concentrations usually exerted toxic effects on cells. Lower concentrations elicited either a marked loss of activity or were ineffective. Cytochalasin B at 0.3 μg/ml still affected macrophages by 56.7% ± 12.0 (3 tests). Sometimes the activity of a drug remained constant over a wide range of concentrations, as for phenylbutazone and colchicine where the inhibitory effect was unaltered at 100, 10 and 1 μg/ml. Similarly, concanavalin A inhibited chemotaxis at an almost constant rate over the range of 30, 10 and 3 μg/ml.

Discussion. The data considered in this study were obtained in 25 tests. The variation in the absolute numbers of migrated cells in the positive controls was very large: 91.2 ± 52.7 (SD). This means that the migratory capacity of the rabbit macrophages differed widely from one exudate to another. This is in contrast with highly reproducible results obtained with mouse peritoneal cells[7]. On the other hand, the inhibitory or non-inhibitory effect of a drug was more reproducible because this effect occured independently of the number of migrated control cells and was expressed as a percentage of the positive controls.

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In the present investigations we have measured inhibition of chemotactic migration. Because of the difficulties in the assessment of random-migration[8], no attempts were made to distinguish between inhibited cell motility and the ability of the macrophages to sense a chemical gradient. The inhibitory drugs were themselves not chemotactic. The design of the experiment only allows evaluation of the direct effect of a drug on the macrophage; a possible interference of a drug with casein appears highly unlikely. The results are therefore directly comparable with those obtained previously with polymorphonuclear leukocytes from rabbit exudates[1]. Major differences in the effect of some substances on these two types of leukocytes were observed. Phenylbutazone and Tween 80 markedly inhibited chemotactic migration of mononuclear but not that of polymorphonuclear cells. Sodium salicylate and colchicine exerted a more pronounced effect on macrophages than on polymorphs. The other agents tested affected the activity of both cell types similarly.

Hydrocortisone succinate completely failed to affect in vitro chemotaxis of mononuclear cells. This finding contrasts with the results of RINEHART et al.[9] who reported that hydrocortisone (16 to 500 µg/ml) greatly affected the in vitro chemotactic response of human monocytes but not that of polymorphs. The work of RABINOVITCH and DESTEFANO[10] using a different test system to assess macrophage migration (macrophage spreading in vitro induced by subtilisin) corroborates our results concerning impairment of macrophage migration by phenylbutazone, colchicine and cytochalasin B. Furthermore, they found that indomethacin at 50 µmol inhibits macrophage spreading by 50%. This concentration is about 18 µg/ml, which in our assay produced visible toxic alterations of cells within 5 h of incubation. The present results with concanavalin A confirm those of KUMAGAI and ARAI[11] who showed inhibition of guinea-pig macrophage migration from capillary tubes.

The mechanism of action of phenylbutazone and sodium salicylate in inhibiting the migration of macrophages but not that of polymorphs is not understood and remains difficult to explain in view of the ineffectiveness of the other antiinflammatory agents tested. Colchicine and vincristine are known to disrupt microtubules and cytochalasin B affects contractile microfilaments thereby modifying several cellular functions[12]. Studies of BANDMANN et al.[13] suggest that centriole-associated microtubules are essential structures in direction-finding or in the directional movement involved in the chemotaxis of polymorphonuclear leukocytes but not actually in the mechanism of locomotion itself. The two plant mitogens phytohaemagglutinin P and concanavalin A may interact with cell surface receptors and membrane flexibility as reported for lymphoid cells[14]. However, they do not cause cell aggregation in the concentrations used. Interference with glycolysis and chelation of bivalent anions also impair cellular movement as shown by iodoacetic acid and dipotassium EDTA. Tween 80 possibly modifies the cell membrane structure of macrophages rather than that of polymorphs; the inhibition observed may well represent a deleterious effect on cellular functions, though cell damage was not microscopically apparent.

As macrophages are abundantly present in many chronic inflammatory diseases, the ability to influence their chemotactic accumulation may eventually induce beneficial therapeutic effects. These mononuclear phagocytes are attracted by several factors such as serum and complement derived components and a chemotactic lymphokine derived from cell-mediated immune reactions[15]. Once macrophages are involved in the inflammatory process, they exhibit many features characteristic of activated cells including the generation of new inflammatory mediators and the abundant release of lysosomal enzymes into the extracellular compartment[16]. As a single dose of hydrocortisone acetate to infected mice has been shown to greatly delay macrophage accumulation at infective foci[17] and DI ROSA et al.[18] have reported marked suppression of mononuclear migration following treatment of rats with several antirheumatic drugs, the failure of the antiphlogistic drugs to influence leukocyte chemotaxis in vitro may imply that the assay presently employed does not adequately reproduce in vivo conditions. To support this assumption, we have been able to demonstrate effects of substances which were previously shown to be inactive on neutrophils in vitro[1] by studying localized leukocyte mobilization in the ears of rabbits previously given the antiphlogistic orally[19] (FEURER and BOREL, unpublished results).

Summary. Chemotaxis of rabbit macrophages was inhibited in vitro by phenylbutazone and sodium salicylate, but not by other antiinflammatory agents. Other inhibitory compounds were colchicine, vincristine, PHA, Con A, iodoacetic acid, cytochalasin B, and EDTA. Some of these in vitro results contrast apparently with in vivo effects.

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