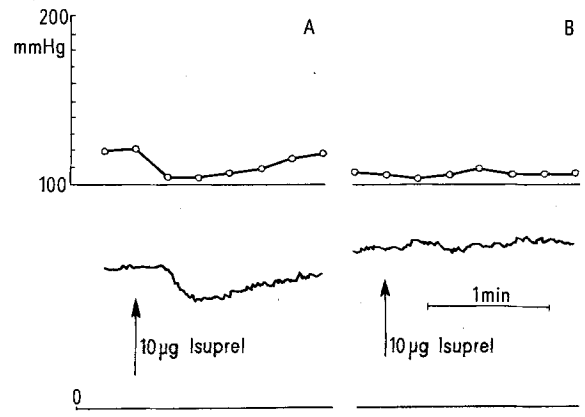

Fig. 1. Effect of isoprenaline before (A) and after (B) 0.3 mg/kg propranolol administration. 10 kg dog. Above: mean arterial blood pressure. Below: local flow of cerebral cortex.

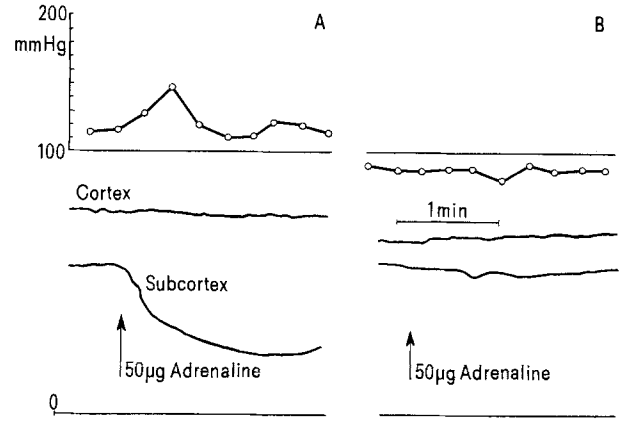

Fig. 2. Effect of adrenaline before (A) and after (B) 0.3 mg/kg phentolamine administration. 24 kg dog. From top to bottom: mean arterial blood pressure, local flow of cerebral cortex and subcortical white matter. In panel A) blood pressure increase was damped with a pressurized chamber connected to the arterial system.

Results. The results obtained by direct blood flow determinations are summarized in the Table. β-adrenergic stimulation by isoprenaline administration failed to induce any significant change in the cerebral haemodynamics (I). Adrenaline, a stimulator of both α- and β-activities induced in the cerebral vascular bed pure vasoconstriction (increase of resistance), which was completely prevented by phentolamine, an α-blocking agent (II). Phentolamine pretreatment did not reveal any masked dilator action of the adrenaline (II/B), nor was it revealed by selective for β-blockade induced by propranolol, since after the latter drug, adrenaline administration failed to elicit an accentuated increase in cerebral vascular resistance (III). Finally, β-blockade proved to be ineffective in modifying the well known enormous dilator effect of asphyxia on cerebral vasculature (IV). This excludes the possibility that β-adrenergic influences contribute to the metabolic adaptation of the vessels in the brain.

Results of the measurements of the local flow changes were consistent with the above data. Not a single experiment revealed true dilator effects after any types of adrenergic stimulation. Figure 1 shows the typical effect of isoprenaline: on administering the drug, a flow decrease ensues in the cortical grey matter, which is roughly proportional to the concommittant slight blood pressure drop. On the contrary, α-adrenergic stimulation often resulted in a regional constriction which could be abolished by α-blokade. As seen in Figure 2, there was a greater chance to obtain a clear constrictor response in the subcortical white matter than in the cortex.

Comments. On the basis of the present pharmacological analysis, the vascular bed of the brain appears to represent a typical α-region. This concept corresponds well with that recently described for the neural control of the cerebral vessels[2]. In most cases α-adrenergic constriction resulted in a mere increase of calculated vascular resistance without an appreciable flow decrease as far as the overall flow response is concerned. However, α-adrenergic stimulation does not affect the entire cerebral vascular system uniformly: by measuring local tissue heat clearance an α-sensitive decrease in flow was frequently found, especially in the subcortical regions, despite the simultaneous increase of the arterial blood pressure. In the cortex this type of response was rare. Evidently, the more pronounced metabolic demand of the cortical cells was able to mask adrenergic constriction too soon and too easily in the latter region. On the other hand, all attempts to induce beta-adrenergic dilatation in the brain proved to be unsuccessful. Moreover, as hypoxic dilatation did not change significantly after propranolol administration, β-influences could not play an important role in the metabolic adaptation of the vessels.

Zusammenfassung. Die Wirkung der Katecholamine ruft in der zerebralen Durchblutung eine Vasokonstriktion hervor, die mittels α-Blockierung behoben werden kann. β-dilatatorische Wirkung ist an den zerebralen Gefässen nicht nachweisbar.

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Effect of Some Drugs on the Chemotaxis of Rabbit Neutrophils in vitro

Introduction, There is strong evidence that cellular accumulation in inflammatory reactions is due to chemotactic and not random migration of leukocytes[1–4]. Inhibition of leukocyte chemotaxis by drugs has been studied by several workers, yielding highly diverging and not directly comparable results. The present study includes 20 drugs which could be suspected of interferring with chemotactic migration. The experimental design allows the detection of only a direct action of the drug on the cell. We have looked for neither inhibition of cytotaxin formation nor inactivation of already formed cytotaxins.

Methods. The Boyden chamber method for the in vitro evaluation of chemotaxis was used[5,6]. The cells used throughout these experiments were derived from rabbit peritoneal exudates induced by the injection of 3% sodium caseinate 2.5 h before collection and contain-

ing 95 to 100% neutrophils. The cell suspension was standardized to 4×10^6 cells per chamber and the granulocytes, suspended in modified Gey's solution (10 mg glucose/ml), were preincubated with the drug for 20 min at 37 °C. Thereafter the mixture of cells plus drug was transferred into the upper compartment of the Boyden chamber, the lower compartment of which had just been filled with 5% normal rabbit serum in Gey's solution activated with an immune complex (2% HSA-anti-HSA) as cytotaxin. The incubation period in the chamber was 2.5 h. A single filter technique with 3 µm Millipore filters was used[7].

A suitable solvent for each drug had to be found and each substance was assessed in several dilutions for its cytotoxicity on Cr^{51}-labelled rabbit exudate neutrophils. A Cr^{51} release of $\leqq 5\%$ above controls was considered as the toxic limit. In a range of only 2–5% above control values, the toxic effects were doubtful and in the Table those concentrations are preceded by the sign $\geq$. The compounds were subsequently tested in non-toxic doses for inhibition of chemotaxis. Despite numerous experiments, some drugs like hydrocortisone succinate, Na-salicylate and colchicine produced such variable effects that it remains questionable whether or not they inhibit chemotaxis.

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Substances tested for inhibition of rabbit neutrophil chemotaxis in vitro

Drugs tested	Solvent[a]	Minimal toxic level for neutrophils µg/ml	Chemotaxis test in vitro			
			Concentration µg/ml	Number of migrated cells; treated cells as % of controls (Mean ± S.E.)	No. of tests *n*	Inhibition
Anti-inflammatory drugs						
Hydrocortisone (Sigma)	Tw/DMSO	100	10–3	92.2 ± 8.4	6	—
Hydrocortisone succinate (Upjohn)	Gey's	> 100	100	84.5 ± 6.1	6	?[b]
Dexamethasone (Ciba-Geigy)	Tw/DMSO	10	3–1	106.1 ± 12.6	13	—
Indomethacine (Merck)	Tw/DMSO	10	3–1	90.9 ± 10.7	12	—
Phenylbutazone (Ciba-Geigy)	$NaHCO_3$	> 100	100	98.6 ± 10.5	5	—
Naproxen (Syntex)	$NaHCO_3$	≥1000	100	108.8 ± 16.2	5	—
Na-salicylate (Merck)	Gey's	>1000	1000	59.9 ± 17.0	8	+/?[b]
Inhibitors of cell division						
Demecolcine (Ciba-Geigy)	Gey's	≥ 100	1	35.5 ± 8.2	8	+
Colchicine (Sandoz)	Gey's	≥ 100	100	60.3 ± 11.9	6	+/?[b]
Vincristine (Lilly)	Gey's	>1000	10–3	29.9 ± 4.9	7	+
Actinomycin D (Serva)	Gey's	> 10	10	98.5	1	—
Mitogenic agents						
Phytohaemagglutinin P (Difco)	Gey's	> 100	25	12.1 ± 6.1	7	+
Concanavalin A (Calbiochem)	Gey's	≥ 300	10	14.8 ± 4.9	5	+
Agents interfering with glycolysis						
Iodoacetic acid (Merck)	Gey's	≥ 100	10	11.7 ± 3.6	9	+
Cytochalasin B (Sandoz)	DMSO	≥ 30	0,3	17.5 ± 5.8	4	+
2-deoxyglucose (Fluka)	Gey's	>1000	8000[c]	94.2 ± 11.4	5	—
Miscellaneous						
Dipotassium ethylenediamine-tetracetate (Fluka) (chelating agent)	Gey's	≥2000	1000–100	39.3 ± 6.3	4	+
Adenosine-3′5′-cyclic monophosphate (Fluka) (effect on motility?)	Gey's	> 100	100–3	93.4 ± 8.1	5	—
Chloroquine (Bayer) (lysosomal stabiliser)	Gey's	≥ 10	10	90.7 ± 8.6	6	—
Trypsin (Difco) (proteinase)	Tris	≥2000	2000	123.0 ± 20.0	3	—

[a] Solvents: Gey's solution was used as diluting solution. Tw/DMSO, 0.02 ml Tween 80 plus 0.1 ml DMSO (dimethylsulfoxide) used as solvent for 1 mg substance; DMSO, 1.0 ml dimethylsulfoxide for dissolving 1 mg substance; $NaHCO_3$, 0.1 ml 5% sodium bicarbonate for dissolving 1 mg substance. *Tris*-buffer (pH 7.2) used as diluent for trypsin. [b] These substances showed inconsistent results ranging from no inhibition at all to weak or sometimes intermediate inhibition. [c] Only 1 mg glucose/ml Gey's solution instead of 10 mg/ml was used in these tests.

Summary indicating source and known effect of substance, solvent used, lowest cytotoxic concentration for neutrophils and results of the chemotactic experiments. Each test included one to several concentrations of a substance, each dilution being always assayed in triplicate. The average number of control cells (± S.D.) having migrated through the entire thickness of the filter in the 61 tests included in this Table was 103 ± 76 cells per high power field. The numbers of migrated cells incubated with substance are expressed as percentage of the controls. The average percentage from *n* experiments using the drug concentration indicated in the previous column is shown for each compound with the standard error of the mean. Tests performed with other concentrations are not considered here.

Results. The results are summarised in the Table. None of the antiinflammatory drugs clearly inhibits neutrophil chemotaxis. The inhibitors of cell division demecolcine, vincristine and to a limited extent colchicine, but not actinomycin D, exert a clear inhibitory effect on cell migration. The complete inhibition of neutrophil chemotoxis by 2 mitogenic substances, phytohaemagglutinin P and concanavalin A, is noteworthy. In the group of agents interfering with glycolysis, iodoacetic acid and cytochalasin B inhibit chemotaxis of rabbit neutrophils. Even when tested in a ratio of 8 mg 2-deoxy-glucose: 1 mg glucose per ml, the non-metabolisable 2-deoxy-glucose produces no significant diminution of cellular migration in vitro. Among the miscellaneous drugs, only dipotassium EDTA consistently shows a weak to intermediate inhibition of migration. Cyclic AMP, chloroquine and trypsin do not affect chemotaxis.

Discussion. The Boyden chamber represents an in vitro model suitable for measuring leukocyte chemotaxis and does not adequately reproduce in vivo conditions. Therefore, some of the substances tested may act at a different level than that investigated with the present technique. Our experimental data suggest that anti-inflammatory drugs do not primarily interfer with cell migration[8] and that drugs acting at the level of chemotactic migration are still to be discovered. KELLER and SORKIN[9], using the same Boyden technique, have found hydrocortisone, prednisolone and phenylbutazone to have no inhibitory activity against granulocyte chemotaxis. MOWAT and BAUM[10] observed that incubation with hydrocortisone in vitro of polymorphonuclear leukocytes from patients with rheumatoid arthritis fails to alter their chemotactic behaviour. PETERS et al.[11] found no significant effect on the localised mobilisation of leukocytes from patients treated with hydrocortisone. However, KETCHEL et al.[12] observed inhibition of the amoeboid migration of human leukocytes when hydrocortisone was added in vitro. WARD[13,14] reports that hydrocortisone, methylprednisolone, phenylbutazone, and chloroquine are all strong chemosuppressants of leukocytes. Although PHELPS[15] has claimed that indomethacine inhibits the random motility of polymorphonuclear leukocytes, our results suggest that this compound is inactive for directed migration.

The mechanism for migration inhibition by demecolcine, colchicine, and vincristine is at present not understood[16,17]. It is known that these 3 compounds affect the process of cell division by preventing formation of the mitotic spindle, while actinomycin D has a different mechnism of action. Our results with demecolcine, colchicine and vincristine agree with those of WARD[13]. CANER[18] also confirmed that colchicine inhibits leukocyte migration in vitro. However, PHELPS[19] discovered that colchicine suppresses the formation of the chemotactic substance released by granulocytes after phagocytosis of urate crystals.

The mechanism by which mitogenic agents interfere very effectively with granulocyte migration remains obscure. The two substances tested have also shown a strong activity in the target cell destruction test, where they markedly inhibit the cytotoxicity of sensitised spleen cells[20].

Inhibition of chemotaxis can be achieved by iodoacetic acid, an agent affecting glycolysis, and by cytochalasin B, which interferes with glucose transport into cells[21,22], but not by 2-deoxyglucose. The present results with iodoacetic acid confirm those obtained by CARRUTHERS[23] with human leukocytes. Cytochalasin B inhibits reversibly chemotaxis of rabbit neutrophils[24], a finding also reported by BECKER et al.[25] for both rabbit and human polymorphonuclear leukocytes. Moreover, it is questionable whether cytochalasin B influences cell movement by inhibiting the glucose transport into the cell or by interfering directly with the contractile microfilaments[26].

The somewhat weak inhibition exerted by the chelating agent EDTA seems to be due to depletion of free bivalent anions needed for motility. Cyclic AMP as well as dibutyryl cAMP (data for the latter not shown here) neither possess activity inhibitory to cell motility nor act as chemotactic agents[27,28].

Many aspects of the interaction of drugs with the mechanism of chemotactic cell migration are not yet understood. Such studies may contribute to the control of chemotaxis in inflammatory reactions[29].

Zusammenfassung. Untersuchungen über den Einfluss 20 bekannter Substanzen auf die chemotaktische Wanderung von Kaninchenexsudatzellen, vorab Neutrophilen, werden dargelegt. Im Chemotaxistest nach Boyden wurden die Leukozyten zusammen mit Substanz inkubiert und die Veränderung ihrer gerichteten Wanderungsfähigkeit gemessen. Die Bedeutung der Ergebnisse werden kurz besprochen.

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