tration of FMLP in the outer well. By 2-way analysis of variance FMLP had a significant effect on chemotaxis ($p < 0.01$). Comparison of the responses of 10^{-6} M colchicine treated cells with those of control cells by paired T-test failed to demonstrate any significant differences in any gradient ranging from a ratio of 2:1 to 20:1. Similar results were obtained for 10^{-7} and 10^{-8} M colchicine (data not shown).

Colchicine has been postulated to inhibit chemotaxis by disrupting the assembly and disassembly of microtubules[3]. The evidence for this hypothesis is based mostly on experiments utilizing micropore filter chambers. In those studies, cells treated with 10^{-6} M colchicine show up to 75% inhibition of chemotaxis[3,4]. Random migration, either spontaneous or stimulated under gradient free conditions, is largely unaffected. Evidence of the proposed defect in orientation has come from the ultrastructural studies of Malech[3] in which the ability of cells to respond to rapid reversal of the gradient was impaired by treatment with 10^{-6} M colchicine. The impairment was manifested by a loss of orientation of the centriole relative to the nucleus accompanied by a significant decrease in the number of centriole associated microtubules. Zigmond, however, has reported that colchicine-treated cells observed in a direct visual assay were still able to orient in a gradient[12]. Using a filter assay, Valerius[13] also found that colchicine-treated cells could orient in a gradient, but activated random migration was decreased.

Our results indicate that locomotion itself is unaffected by colchicine. Neither spontaneous nor activated random migration was significantly affected by the drug. Furthermore, the responses of the cells in varying concentrations and gradients of FMLP failed to show any impairment of chemotaxis. We conclude that colchicine, at therapeutic serum concentrations in vivo, does not affect either locomotion or chemotaxis.

The reasons for the discrepancy between our results and those observed in micropore filter systems are unclear. A major difference between the agarose and micropore filter assays is the need for cell deformability in the latter. In filter assays the cells are required to pass through a tortuous network of fibres in order to penetrate the membrane. In the agarose system the cells can move freely along the surface towards the source of the gradient. Indeed defects of cell deformability occurring either spontaneously, or following various in vitro treatments, correlate well with defective chemotaxis in micropore filter[15] assays. In conclusion, our data indicate that colchicine at tested concentrations, had no effect on cell locomotion using the agarose technique. These findings are in agreement with the conclusion of Valerius that microtubular assembly is not required for PMN orientation[13], and suggest the possibility that the effects of colchicine on PMN locomotion in filters may occur at other sites in the cell.

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IgE rheumatoid factor

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Summary. A solid phase enzyme-linked immunosorbent assay for detecting class-specific rheumatoid factors (RF) has been devised. IgE RF, which has not been reported yet, was found in sera from 3 of approximately 100 rheumatoid patients.

Rheumatoid factor (RF) activity is found in IgM, IgG, IgA and IgD[1]. However IgE RF has not been reported. We devised an enzyme-linked immunosorbent assay (ELISA) for detecting class-specific rheumatoid factors (RF). Measuring approximately 100 samples from patients with active rheumatoid arthritis (RA), we found a case of pleuritis-associated RA in which a high level of IgE-RF was observed both in the serum and in the pleural effusion. A moderate elevation of serum IgE RF was found in another 2 cases, but approximately 200 sera from normal subjects and patients with RA and SLE were negative. The ELISA was based on the solid phase sandwich method, and our previous method[2] for measuring serum IgE levels was employed with some modifications.

Polystyrene tubes were coated with rabbit IgG (RIgG) instead of anti-IgE antibody and a horseradish peroxidase (HRPO)-labelled Fab fragment of anti-IgE antibody was used in place of HRPO-conjugated anti-IgE whole antibody to avoid the interference of RF other than IgE RF[3]. RIgG which also reacts with RF was used instead of human IgG in order to avoid the reaction with antihuman IgG antibody for detecting IgG RF. When we used anti-IgE whole antibody in place of Fab fragment, a weak false positive reaction due to the binding between all classes of RF and Fc portion of the antibody was observed. Specific anti-IgE antibody (anti-ε) was prepared as described in the previous paper[2].

In order to determine the quantity of IgE RF in the patient,

the serum and pleural fluid were absorbed 4 times by RIgG or human IgG coated tubes to remove immunoglobulins possessing RF activity. Total IgE levels before and after the absorptions were measured by ELISA using the solid phase sandwich technique[2] with a slight modification. HRPO labelled antiIgE Fab was used instead of HRPO-antiIgE whole antibody, and BSA was used in place of rabbit serum. Total IgE concentration in the serum and pleural fluid decreased markedly after the first 3 absorptions and was not changed by the 4th absorption. The results indicated that 330 ng/ml (66%) of IgE in the serum and 650 ng/ml (78%) of IgE in the pleural fluid had RF activity, whereas the total IgE concentration in the serum from a asthmatic patient did not change after the absorption by RIgG coated tube.

The patient with RA was treated with prednisolone and arthritis and the pleural effusion subsided. However, the effusion accumulated again with a decrease in the dose of prednisolone. Sera were obtained from the patient at various intervals and the levels of IgE-, IgG-, IgM-RF were measured. It was observed that the serum levels of IgE RF were more parallel with the disease activities than those of IgM and IgG RF. Therefore we think that IgE RF might have played some role in this patient.

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Penetration of regenerated neurosecretory fibres into the saccus vasculosus following hypophysectomy in the catfish, *Heteropneustes fossilis* (Bloch)

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Summary. Regenerating neurosecretory fibres were traced in 150-day post-hypophysectomized catfish, *Heteropneustes fossilis*. The regenerated neurosecretory fibres reorganised into a neurohypophysis-like structure, as well as extending several neurosecretory fibres into the epithelium of the saccus vasculosus, and a few into its lumen. This circumventricular organ may serve as a cellular bridge between the cerebrospinal fluid and the vascular system.

Studies on the hypothalamo-neurohypophysial complex of fishes following hypophysectomy are few[2-9], and have generally described changes in the neurons of the nucleus preopticus and neurosecretory material. The fate of neurosecretory fibres other than those that terminate in the neurohypophysis-like structure has not been described. Neurosecretory fibres are shown here as they regenerated in the catfish, *Heteropneustes fossilis*, 150 days after hypophysectomy.

Material and methods. 25 catfish were hypophysectomized and 25 were sham-operated after acclimatisation to laboratory conditions. Sexes were not separated since preliminary investigations indicated no sexual distinctions in the hypothalamo-hypophysial system. Each fish was anesthetized by immersing in 0.034% aqueous solution of methane tricaine sulphonate, and the parasphenoid bone at the level of the pituitary was drilled with the help of a fine burr. The exposed pituitary was sucked out using a glass pipette. Sham-hypophysectomy was performed in the same way, but the pituitary was not disturbed. 8 hypophysectomized and an equal number of sham-operated and unoperated controls were sacrificed 150 days after operation, following the conclusion of other studies. Brains were fixed in Bouin's fluid, and 4–6-μm-thick sections were cut in the sagittal and transverse planes. They were stained with alcian blue, aldehyde fuchsin and chrome alumhematoxylin-phloxin.

Results. In unoperated controls and sham-operated fish the axons of the aldehyde fuchsin-positive neurons of the preoptic region extend ventrolaterally, curve posteriorly, unite ventromedially and enter the stalk of the pituitary gland (figure 1). Neither the basal hypothalamus posterior to the stalk nor the saccus vasculosus show any aldehyde fuchsin-positive fibres.

In the hypophysectomized fish neurosecretory fibres severed at the stalk regenerated and reorganised to form a neurohypophysis-like structure (figures 2 and 3). Some of the neurosecretory fibres penetrate the ependyma and project into the 3rd ventricle. Several fibre bundles extendn posterior to the stalk and ventral to the 3rd ventricle as a thick aldehyde fuchsin-positive fibre tract. The axons contain deeply stained neurosecretory granules. The main bundle of regenerated peptidergic fibres extends to the saccus vasculosus; then it ramifies and the branches enter its wall. In the anteroventral region of the saccus vasculosus neurosecretory fibres enter and encircle small lobules of epithelial cells. Some fibres terminate around the capillaries in the epithelium of the saccus vasculosus. Neurosecretory fibres also penetrate the epithelial wall and project into the central lumen of this circumventricular organ.

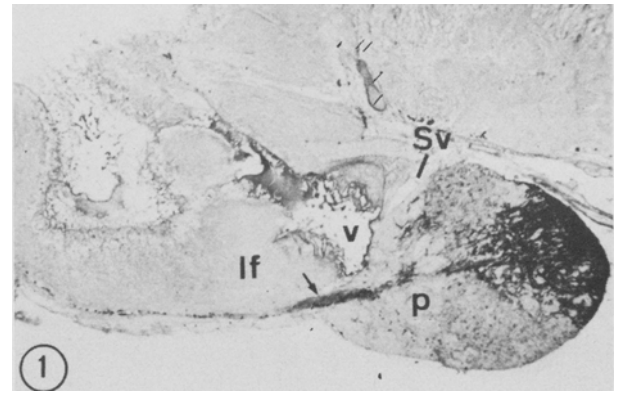

Fig. 1. Sagittal section of the brain of sham-operated catfish, *Heteropneustes fossilis* showing the pituitary gland (p) and saccus vasculosus (Sv). Arrow shows the neurosecretory fibre tract which enters the hypophysis through the pituitary stalk. Also note the absence of neurosecretory fibres posterior to the stalk. If, Infundibulum; v, 3rd ventricle. × 128.