blue, and placed on a microscope slide. In both methods the mast cells were counted in an area of 10 mm², and the average mast cell count per 1 mm² was calculated.

Results and discussion. As the results obtained from the control material show, both the methods give the same result as far as untreated mast cells are concerned. It is also noted that, if there are differences in the mast cell counts obtained with both methods, these differences must be regarded as changes in the heparin or histamine content of the cells.

As expected, rapid disruption of the mast cells occurred, and only a few traces of mast cells could be found in the specimens up to 16 days. Thereafter new small mast cells began to appear in the specimens; that is, regeneration had begun. At 16 days the number of mast cells demonstrable with histamine stain was only about half the number of cells visible with metachromatic staining. Later the difference decreased. The count of the cells which became visible with toluidine blue reached the control level at 32 days. On the other hand, the number of the cells stained with Reinecke-salt was still only 80% of the control at 64 days.

The results point to the possibility that the bond between the histamine and heparin in the mast cells is not definitely fixed and that the amounts of the heparin and histamine in the mast cells may vary within certain limits. There are two explanations for the fact that the mast cell count obtained with histamine staining is smaller than the count obtained with toluidine blue staining. It may be possible that the effect of compound 48/80 lasts for a very long time. The second and more probable explanation is that the young and regenerating mast cells always have a lower concentration of histamine than full-grown mast cells.

The experimental conditions in this study were rather pathological. Whether the mast cells can vary their histamine and heparin content physiologically as well is a fundamental problem. This function is probable if we take into consideration their manifold role in the connective tissue.

Zusammenfassung. Der Histamin- und Heparingehalt in Mastzellen, die nach totaler Zerstörung mit Histaminliberator 48/80 regenerieren, wird untersucht. Die Ergebnisse zeigen, dass während des Regenerationsprozesses die Anzahl der Mastzellen, in denen sich das Histamin mit Reineckesalzlösung anfärbt, kleiner ist, als die Anzahl der Mastzellen, die mit Toluidinblau darzustellen sind.

E. VALTONEN, B. KOCK,
M. SIIMES, and J. JÄNNE

Department of Anatomy, University of Helsinki (Finland), May 11, 1964.

Catheptic Activity in the Cerebral Tissue of the Rabbit during Allergic Encephalomyelitis

The proteolytic enzymes of the cerebral tissue in normal and pathological conditions have so far been relatively little studied. In 1931 KREBS[1] found in the cerebral tissue a marked peptidasic and a reduced gelatinasic activity. EDLBACHER et al.[2], and later KIES et al.[3], confirmed the existence of proteinasic activity in the brain. The accurate researches of ANSELL and RICHTER[4] proved the existence of a proteinase with optimal pH 7.4, and of a cathepsine with optimal pH 3.5, in fresh cerebral tissue. As proved by several investigations[5,6], in demyelinizing processes, the state of equilibrium between the synthesis and the breakdown of proteins is modified. It may be presumed that these changes are also reflected by the enzymatic activity.

In our experiments, 30 rabbits were treated with an encephalitogenic emulsion, containing antipertussis vaccine, cattle brain homogenate, and Freund's adjuvant, inducing allergic encephalomyelitis[7]. The catheptic activity in the cerebral tissue homogenate (1:4 in isotonic sodium chloride solution) was determined at pH 5.0, with azocasein substrate, in the presence of 25% urea, and 0.05 *M* cysteine. The rate of proteolysis was expressed in μg of the azocasein, hydrolysed by 100 mg wet tissue, after 1 h incubation at 38°C. In the controls, an appreciable catheptic activity was observed, which remained practically unchanged after 10 days, i.e. after 2 injections of antigen. After 3 or 5 injections, i.e. after 20 or 32 days from the beginning of treatment, in the evolutive phase of the pathological process, the catheptic activity showed a significant increase, amounting to 88%, as compared to the controls (see Table).

The increase of the enzymatic activity might be in connection with the allergic inflammatory process of the brain. Recent investigations[8] demonstrated that, in anaphylactic and allergic reactions, the proteolytic enzymes

Mean values of the catheptic activity of the brain in the four groups studied (10 rabbits each)

Controls	Animals treated with encephalitogenic emulsion		
	After 10 days (2 injections of antigen)	After 20 days (3 injections of antigen)	After 32 days (5 injections of antigen)
280 ± 38.2	256 ± 25.8	488 ± 11.1	522 ± 20.5
	$p < 0.001$		

[1] H. A. KREBS, Biochem. Z. *238*, 174 (1931).
[2] S. EDLBACHER, E. GOLDSCHMIDT, and V. SCHLÄPPI, Z. physiol. Chem. *227*, 118 (1934).
[3] M. W. KIES and S. SCHWIMMER, J. biol. Chem. *118*, 616 (1942).
[4] G. B. ANSELL and D. RICHTER, Biochim. biophys. Acta *13*, 87, 92 (1954).
[5] GR. BENETATO, E. GABRIELESCU, L. PARTENI, A. BORDEIANU, and I. BOROŞ, Fiziol. norm. şi patol. (Bucharest) *7*, 73 (1961).
[6] A. EPERJESSY, T. FESZT, V. BLAZSEK, and A. KISS, Orvosi Szemle (Tg. Mureş) *9*, 417 (1963).
[7] D. MISKOLCZY, F. GYERGYAY, and T. FESZT, Z. ges. exp. Med. *137*, 82 (1963).
[8] G. UNGAR, T. YAMURA, J. B. ISOLA, and S. KOBRIN, J. exp. Med. *113*, 359 (1961).

are activated. However, we should not neglect the fact that the increase of proteolysis results from tissular necrotic processes. We ascribe a role to the increase of the enzymatic activity in the development of the pathological process.

Zusammenfassung. Gehirnhomogenate von Kaninchen mit experimenteller allergischer Encephalomyelitis weisen eine deutlich erhöhte katheptische Aktivität auf, wahrscheinlich als Folge des allergischen Entzündungsprozesses.

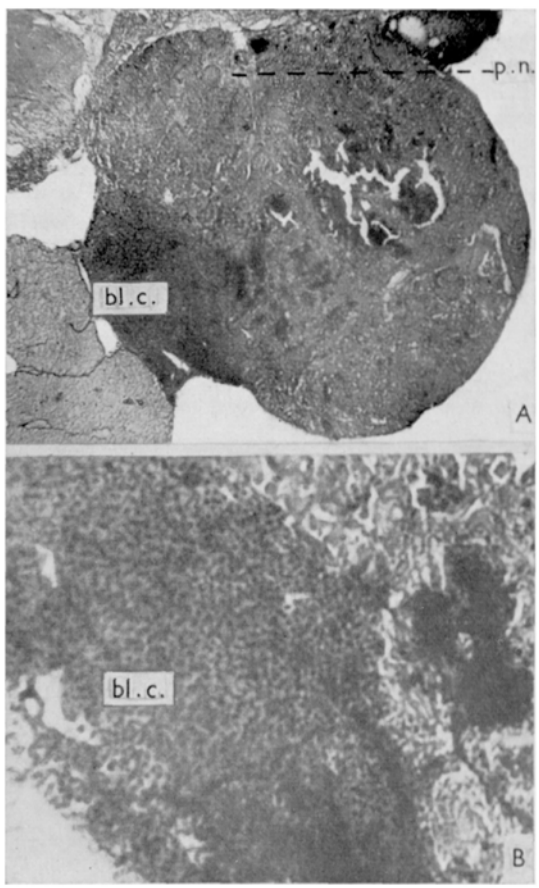
M. F. KEREKES, T. FESZT, and A. KOVÁCS

Research Station of the Academy of Sciences of the Roumanian People's Republic, Tîrgu-Mures (Roumania), July 29, 1964.

Extravasation of Blood Cells in and around the Pituitary of *Anabas scandens* (Cuvier)

PICKFORD and ATZ[1] in their extensive review on the physiology of the pituitary gland of fishes have cited very few cases of abnormality of fish pituitary. Recently, SATHYANESAN[2] has reported an instance of abnormality in the pituitary of *Chela bacaila*.

The pituitary of *Anabas* is compact and essentially of a platybasal type devoid of any definite stalk from the brain. Neurohypophysis is well developed and its extensive ramifications divide the gland into conventional parts, namely pro-, meso-, and meta-adenohypophysis. Pituitary collected from a specimen in April 1963 was fixed in Bouin's fluid and sections of 8 μ thickness were stained in Gabe's paraldehyde fuchsin as modified by DAWSON[3]. The majority of gland cells, especially the basophils, exhibited a marked affinity with paraldehyde fuchsin and very little reaction to other dyes in the counter stain. Pools of blood cells were observed within as well as outside the gland (Figure A). From a pool of free blood cells close to the posterior region of the pituitary, finger-like capillaries were found extending into the gland. The distribution of blood cells was not restricted to any region of the pituitary but a greater concentration of blood cells was noticed in mesoadenohypophysis (Figure B).



Photomicrographs of the pituitary of *A. scandens* showing (A) the distribution of blood cells. A portion of the same (B) enlarged to show clearly the free blood cells. bl.c. – Blood cells; p.n. – Pars nervosa. Bouin/Fuchsin paraldehyde. × 125.

Since pools of blood cells were observed inside the substance of the pituitary, in addition to their distribution outside, the probability of their origin being due to the rupture of meningeal vessels is ruled out. Extensive vascularization of this kind was quite unusual and never observed in any pituitary under normal conditions.

OLIVEREAU[4] reports that the poverty of blood vessels in the mesoadenohypophysis of *Salmo salar* is correlated with the fact that the secretions from this region are discharged in part into the metaadenohypophysis. A greater concentration of blood cells within the mesoadenohypophysis observed in the present case suggests that the secretion from this region enters directly into the blood stream and the occurrence of free blood cells is perhaps due to some unknown pathological condition[5].

Zusammenfassung. Es wurden Gruppen von Blutzellen innerhalb und in der Umgebung der Hypophyse von *Anabas scandens* und besonders in der Mesoadenohypophyse gefunden. Dies fällt auf, da die Mesoadenohypophyse im allgemeinen nicht reich mit Gefässen versorgt ist. Die Gefässversorgung dieser Stelle zeigt, dass das Sekret direkt von der Mesoadenohypophyse ins Blut übergeht, während die Anhäufung von Blutzellen in der Hypophyse pathologisch sein dürfte.

N. H. GOPAL DUTT

Department of Zoology, Annamalai University, Annamalainagar (South India), July 14, 1964.

[1] G. E. PICKFORD and J. W. ATZ, *The Physiology of Pituitary Gland of Fishes* (Zoological Society, New York 1957).
[2] A. G. SATHYANESAN, Naturwissenschaften *6*, 176 (1959).
[3] A. B. DAWSON, Anat. Rec. *155*, 63 (1953).
[4] M. OLIVEREAU, Ann. Inst. Oceanogr. Monaco *29*, 95 (1954).
[5] *Acknowledgment.* The author is grateful to Professor P. GOVINDAN for guidance and instruction, and to the Government of India for the award of a scholarship.