$ACTH_{4-10}$ to rabbits increased the plasma glucose concentration; this hyperglycemia was associated with a decline in insulin and calcium blood levels. In the rat, neither of these effects was observed. As ACTH is a hormone acting on the adrenal gland, the hyperglycemic effect observed after administration of $ACTH_{4-10}$ could be the consequence of a previous release of cortisol. This is not the case since $ACTH_{4-10}$, in contrast to ACTH, does not stimulate glucocorticoid production. Indeed, in our hands, injections of $ACTH_{4-10}$ up to 150 µg/kg were ineffective on blood cortisol levels. Our observations therefore agree with those of Smotherman et al.[19] who obtained similar results in the rat. Moreover it has been shown by others and by us that this heptapeptidic sequence is inactive on the adrenal gland[4,21,22].

Therefore we show that in the rabbit hypocalcemia, hypoinsulinemia and hyperglycemia are intimately related. In 1968, Curry et al.[23] showed the importance of calcium ions in insulin secretion. It can be suggested that hypocalcemia resulting from the administration of $ACTH_{4-10}$ in the rabbit, or the release of an endogeneous peptide following stress in control animals, inhibits insulin secretion from pancreatic β cells, resulting in hyperglycemia. These results are in agreement with current findings on the role of Ca^{++} in the regulation of insulin release by pancreatic islets. The mechanism by which hypocalcemia could inhibit insulin release is beyond the scope of this short note. However it is clear that the insulin secretory process and the metabolism of glucose is controlled in β cells by calcium handling[24-26]. Several mechanisms have been proposed; an action of the Ca^{++} on enzymes converting proinsulin into insulin[26], an action on the microtubule filamentous system[27], or a modification of the cytoplasmic membrane permeability[26].

The process by which $ACTH_{4-10}$ induces hypocalcemia in the rabbit is not yet clearly understood. However we suggest that $ACTH_{4-10}$ may stimulate the incorporation of calcium in various tissues resulting in a short term hypocalcemia. In agreement with this hypothesis we have demonstrated that $ACTH_{4-10}$ stimulates the uptake of Ca^{++} in bone tissues[22].

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Different neuropathological effects of intrahippocampal injections of kainic acid and tetanus toxin

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Summary. Behavioral and neuroanatomical effects of hippocampal injections of kainic acid (KA) and tetanus toxin (TT) were investigated in rats. Injections of KA resulted in both local and distant neuroanatomical damage, but not in clear signs of epilepsy; injections of TT on the other hand were followed (in some of the rats) by prolonged seizure attacks, but not by neuronal damage. Based on these results it is suggested that the widespread neuronal damage following KA lesions cannot be primarily attributed to orthodromic activation of epileptic discharges. Instead, specific properties of KA and their interactions with certain transmitters may provoke widespread neuroanatomical damage.

Neurotoxic agents are frequently used to study relations between brain damage and behavior[3-5]. Among these agents, kainic acid (KA) has been the most widely-applied substance in recent research. In spite of this fact, ideas on its mechanism in destroying nerve cells are still speculative and controversial[6-13]. The most common hypotheses on the action of KA in the brain are based on an 'excitoxic' action of KA[7,10]: KA is thought to bind to specific receptors and thereby to induce an over-excitation of the neuron which ultimately may result in its destruction.

Because in experiments with KA a high correlation exists between the presence of epileptic discharges and the de-

struction of neuronal tissue both at the injection site and in certain regions distant from it, it is assumed that the duration of seizure attacks is indicative of the amount of destroyed nervous tissue[14]. Schwob et al.[11] (p. 992) found 'a better than 90% correspondence between the occurrence of convulsions and the presence of detectable neuronal damage'. Using i.p. injections of ibotenic acid, we have shown that this correlation does not necessarily hold[5]. By comparing the effects of tetanus toxin (TT) with those of KA we shall provide further evidence which challenges the excitotoxic view.

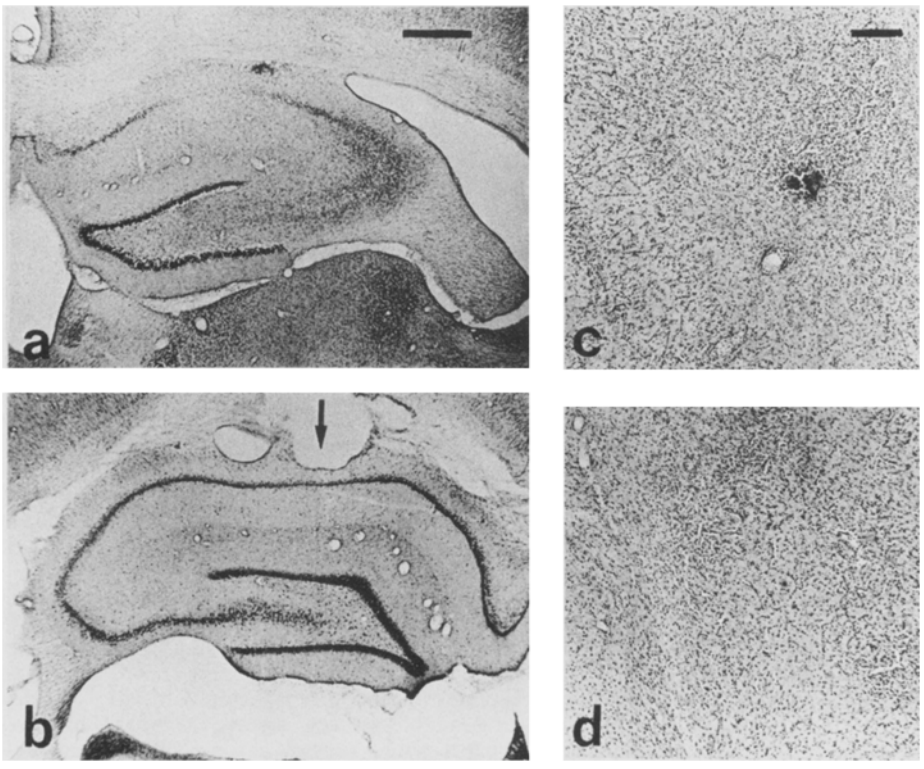
Male Sprague-Dawley rats of about 200 g received under anesthesia either KA (n = 2) or TT (n = 8) injections in the dorsal hippocampus, using techniques described previously[4,15]. Rats of group KA received an intrahippocampal injection of 1.3 nmoles KA. 1 rat of group TT received bilateral injections (0.5 µl) of 50 mouse LD_{50} TT per hemisphere (rat A), 3 rats (rats B–D) were injected unilaterally with 50 mouse LD_{50} TT and 4 rats (rats E–H) received a unilateral injection of 10 mouse LD_{50} TT. The original concentration of TT was 1×10^8 mouse LD_{50}/mg. The 4 animals with the high doses of TT survived the operation for 5 days, the other 4 for 4 weeks. KA-injected rats had a 6-day survival time.

Animals were observed throughout their survival times for approximately 8 h per day. Seizure activity and body movements were recorded for time of onset and duration. Following the survival time, the rats were deeply anesthetized and perfused with saline and buffered formalin or a diluted glutaraldehyde-paraformaldehyde mixture. Brains were removed and stored in cold sucrose-formalin solution; sections were stained with cresyl violet and studied carefully under the light microscope.

Epileptic attacks were clearly visible in 4 of the 8 TT-treated rats (rats A–C, E). The 2 KA-injected rats showed some signs which were only indicative of seizure activity (hyperactivity, vibrissae twitching, forelimb clonus), but did not manifest clear tonic-clonic convulsions. Convulsions developed at different times in the TT-treated animals. While rat E (10 mouse LD_{50}) showed epileptic activity only during the last week of its 4-week postinjection-survival time, rats A–C showed convulsions of changing frequency and duration throughout their 5-day postinjection-survival time. In rat E 8 epileptic attacks each of about 30 sec duration were observed during a 4-day period. Rats A–C showed convulsive activity of usually 30–60 sec duration (ictal stage) with an average frequency of approximately 1 attack per 3 h. Rat B, furthermore, developed autoaggression on the 4th postinjection day (biting into its right hind leg).

Anatomically, neuronal damage was detected in KA-treated rats only. Above all, the pyramidal layer of the dorsal hippocampus was almost totally destroyed (fig., a); furthermore, distant lesions were detected in the piriform cortex, amygdala, substantia nigra, mediodorsal thalamus and medial prefrontal cortex (fig., c, d). Despite a careful search, none of the brains of the TT-treated rats manifested any neurological alterations aside from the small zone of damage caused by the insertion of the needle (fig., b).

Though our failure to detect neuronal damage in the TT-treated animals is in accordance with the results of other studies using TT[10,16], this finding appears surprising especially for those 3 rats (A–C) with frequent epileptic attacks. This result is therefore at variance with findings which revealed 'a good correlation between the severity of seizure discharge in the hippocampus and the subsequent pathology'[6] (p. 388; cf. also Ben-Ari et al.[7]). Ben-Ari[6] observed pathological alterations in the hippocampus after only 30 min following the injection of KA. The patterns of neuronal and behavioral alterations appears to be grossly reversed in our 2 groups of rats; KA-treated animals lacked clear signs of seizure activity and revealed, in spite of an only moderately high dose of KA, widespread loci of neuronal damage, while TT-treated rats manifested repetitive convulsions for days with a comparatively high dose of TT (compare our dose with that of Mellanby et al.[17] who



Neuroanatomical changes following injections of kainic acid (KA) and tetanus toxin (TT). *a, b* Appearance of the dorsal hippocampus after injection of KA (a) or TT (b); in *a* the neurons of the CA2–CA4 fields are almost completely destroyed, while in *b* the neurons appear normal though the injection was centered into the area shown (arrow); scale in *a* represents 500 µm and holds also for *b*. *c, d* Distant lesions in portions of the amygdalar (c) and piriform (d) cortex following injections of KA; scale in *c* represents 200 µm and holds also for *d*.

injected only 1 µl of 5–10 mouse LD_{50} TT), but signs of neuronal damage attributable to the toxin were still lacking in our animals.

We conclude from these contradictory effects in KA- and TT-treated animals that an explanation of the widespread neuronal damage following KA-injections by an anterograde propagation of epileptic activity[7,18] is not supported by our data. Duration of seizure activity per se can account for neither the many damaged loci in KA-treated rats, nor the lack of damage in TT-treated rats. Dam et al.[19] have shown with electrical stimulation of hippocampal neurons that even a number of up to 140 induced seizure attacks each of about 50-sec duration may fail to result in neuronal loss in the hippocampus.

Seizure activity may accompany the decay of neuronal tissue in widespread loci of the brain following injections of KA, but apparently it is not its primary cause. This must be sought in the chemical properties of KA itself and in the interaction of KA with specific neurotransmitters (glutamate, aspartate)[9].

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Investigation of *Aeromonas* isolated from water; a serological study using Ouchterlony and immunoelectrophoresis techniques

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Summary. We have shown that immunoelectrophoresis and the Ouchterlony double diffusion technique are valuable assays for the identification of *Aeromonas* species. Serological correlations have been found between the antigenic extracts originated from a collection reference strains of *Aeromonas hydrophila* subsp. *hydrophila* and those originated from wild type water isolated.

To the genus *Aeromonas* belong important fish pathogens responsible for several communicable diseases such as furunculosis; thus, these bacteria may be of economic significance in commercial fish farming[2-4]. Moreover, from an epidemiological point of view, they can be considered as pollution indicator organisms for aquatic environments[5-7]. For these reasons, methods should be developed to allow rapid and reliable differentiation among these bacteria, at the species and possibly subspecies levels. Two serology assays have already been examined by two of us; comparisons between morphological, physiological, biochemical characters and slide or tube agglutination tests have shown that, in the conditions used, these immunochemical methods were not reliable for species identification purposes, mainly because of their lack of specifity[8]. To determine whether the data given by serology can be improved, we assayed two other techniques (Ouchterlony immunodiffusion and immunoelectrophoresis) using soluble antigen extracts. The results are discussed in this paper.

Materials and methods. Bacterial strains. Reference *Aeromonas* strains were obtained from the American Type Culture Collection (ATCC) and the National Collection of Marine Bacteria (NCMB; Aberdeen, Scotland). These were: *A. salmonicida* subsp. *salmonicida* NCMB 1102, *A. hydrophila* subsp. *hydrophila* NCMB 86, *A. hydrophila* subsp. *anaerogenes* ATCC 15467, *A. punctata* subsp. *punctata* NCMB 74.

Wild type *Aeromonas* strains were isolated from fishes; some of them have been used in our previous investigation[8]: 3617/74, 7535/73, 1561/A1/4, 2535/73, 5816/75, 3569/74, 8430/75, 2437/76.

Growth conditions. Aeromonas strains were isolated on Infusion Agar (BBL 11037) containing 5% sheep blood or on Bromthymol Blue Lactose Agar (BBL 93961). After their isolation, the strains were cultured on DST Agar (Oxoid CM 261), on Furunculosis Agar (Difco 0350-01), or on PPLO Agar (Difco 0412-01). Plates were incubated 24 h at room temperature or at 30 °C.