was reported previously[9]. Investigation of the corpora allata ultrastructure with light microscopy (12-day-old bees treated on the 6th day and 25-day-old bees treated on the 15th day) revealed no degenerative changes after precocene treatment. From these experiments it can be concluded that precocene is not acting as anti-JH or as chemical allatectomizer in adult bees.

Egg-laying activity. The queens of 5 free flying colonies were treated topically with 30–200 µg precocene II. Weekly inspection of the brood area revealed no reduction of egg-laying activity in comparison with the acetone controls during a period of 6 weeks after treatment. Also, compared with the controls, no size reduction was observed in the precocene treated colonies. Thus, it can be concluded that the egg viability was not affected by precocene application on the queen.

Mode of action of precocene II in the adult honey bee. Under natural conditions long life span worker bees have low JH titers[9] while short life span is correlated with increased JH titers[10]. Thus the life shortening effect of high precocene doses cannot be interpreted as an anti-JH action. However, it cannot be decided from our experiments whether the life shortening effect of the high precocene dose is due to an anti-feeding action of the drug, followed by disturbance of endocrine processes[22] or results from a general toxic effect. Furthermore it is not clear why queens can survive at much higher doses of precocene II than workers. Our results, together with those of other authors[23], might mean that the honey bees' corpora allata lack the enzymic capability for oxidizing precocene molecules to highly reactive epoxides which are supposed to exert a cytotoxic action on these glands.

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Hyperglycemic effect in the rabbit induced by $ACTH_{4-10}$

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Summary. In the rabbit, a single i.v. administration of $ACTH_{4-10}$ (130 µg/kg) induces hyperglycemia. As $ACTH_{4-10}$ also induces hypocalcemia and inhibits insulin secretion, we suggest that hypocalcemia, inhibition of insulin secretion, and hyperglycemia, are closely related.

Studies of the relationship between structure and activity of adrenocorticotropin (ACTH) have shown that this hormone possesses different biological properties according to its various target cells. Since they share the same receptor in the adrenal gland the sequence $ACTH_{1-24}$ (synacthen) has the same biological action as the whole molecule.

It is known that the peptidic sequences necessary for the binding and/or the biological action are not always the same. The N-terminal sequence 1–10 of ACTH seems to be essential for the biological action, but the 11–24 sequence is necessary for the binding of the hormone on the adrenal target cells[2–5]. As a consequence of this observation numerous authors have reported that the 11–24 sequence, which is biologically inactive, can present an antagonistic effect towards the whole molecule[4,6,7]. According to Beloff-Chain et al.[8] the 18–39 sequence of corticotropin like intermediate peptide (CLIP) should stimulate insulin secretion. On the other hand earlier studies have shown that in the rabbit $ACTH_{4-10}$ and in some instances α, β MSH or related pituitary peptides induce: a) hyperemia of adipose tissue[9], b) increased concentration of free fatty acids (FFA) in adipose tissue, blood plasma, liver and kidney[9], c) lipemia[10,11], d) acute nonkenotic metabolic acidosis[12,13], e) hypocalcemia with hypophosphatemia in rabbit but not in rats[14].

In addition to these effects we show, in this paper, that $ACTH_{4-10}$ induces hyperglycemia and hypocalcemia, and inhibits insulin secretion.

Material and methods. Our experiments were performed on rabbits and rats. 'Fauve de Bourgogne' rabbits weighing 3000–3500 g, and Sprague-Dawley rats weighing 250–300 g, were kept without food for 24 h before the experiment but

had free access to drinking water. The rabbits were divided into 3 groups of 8 animals. The 1st group was injected with 0.2 ml phosphate buffer (pH 6.2) containing 130 µg/kg $ACTH_{4-10}$ (Bachem, Réf: 8688) via the marginal vein of the ear.

The 2nd group of animals received phosphate buffer only (pH 6.2). The 3rd group comprised untreated animals.

Venous blood samples (3 ml) were obtained by puncturing the marginal vein of the 2nd ear every hour for 4 h.

As a control, blood samples were taken from animals of all groups by puncturing the ear vein before any experimental manipulation. The rats were divided into 2 groups of 16 animals. The 1st group received in the tail vein 17 µg/100 g of $ACTH_{4-10}$ in the same phosphate buffer (pH 6.2). The 2nd group was injected only with phosphate buffer (pH 6.2). Four other untreated rats were used as control animals at time 0.

Four animals at a time were killed every hour by decapitation and blood samples collected in dry heparinized test tubes (for blood glucose) and dry test tubes (for Ca and insulin determination). a) Glycemia was determined by a colorimetric and enzymatic method with GOD-PAP kit (Boehringer-Mannheim), b) calcemia by atomic absorption technique using Pye Unicam SP 9, c) insulinemia by radioimmunoassay with a kit provided by C.E.A. (Commissariat à l'Energie Atomique Sorin Biomedica S.P.A.).

Results. In the rabbits $ACTH_{4-10}$ produced a significant hyperglycemia within 3 h. The control animals also showed an increase in plasma glucose although to a lesser degree than in $ACTH_{4-10}$ treated animals (fig. 1). Glycemia increased approximately 120% within 3 h (from 64 to 142 mg/100 ml). In the control animals this increase was only 65%. There was a significant difference ($p < 0.05$) between treated and control animals but none between the control animals and those treated with phosphate buffer only. Therefore, in the rabbits, puncturing the ear vein induced an appreciable hyperglycemia by itself. The time course of this effect in control animals was similar to that observed in the treated ones. This may be due to an endogeneous peptide which is probably released under the stress produced by the puncture. In rats, glycemia of control animals was not different from treated animals and no increase in blood glucose was observed. These differences observed between rats and rabbits are comparable to those observed in studies of the effect of $ACTH_{4-10}$ on blood calcium. Whereas $ACTH_{4-10}$ induced a clear hypocalcemia in rabbit (fig. 2), this heptapeptide has no hypocalcemic effect in the rat[14].

Plasma levels of insulin were decreased in ACTH treated rabbits ($p < 0.05$) (fig. 1). Conversely, rats did not show any similar modification of insulin levels following $ACTH_{4-10}$ injections. We observed that in all animals insulinemia regularly fell during the 3 1st h, increased during the 4th h and then slowly returned to normal values in the treated animals. This effect was greater in treated animals than in the control group. The time course of the effect was similar in both groups and seemed to be related to the variations in glycemia (fig. 1). In parallel experiments we investigated the dose response curve of insulin levels in response to $ACTH_{4-10}$ administration. The threshold for insulinemia was obtained with the administration of approximately 100 µg/kg of $ACTH_{4-10}$ (fig. 3).

A putative nonspecific effect of the peptide was eliminated by the administration of an oxidation product obtained by treating the heptapeptide by performic acid which oxidates the methionine residue (situated in position 4). No significant difference on glycemia was observed compared to the control group in animals treated with such a compound.

Discussion. In rabbits administration of $ACTH_{4-10}$ has been reported to increase plasma FFA levels, fatty liver and kidney lipemia[10,15]. These changes have been shown to be direct and/or indirect consequences of FFA mobilization. In addition it has been clearly demonstrated that hypocalcemia stimulates the release of FFA from adipose tissue[11,15-18].

In the present study we report that administration of

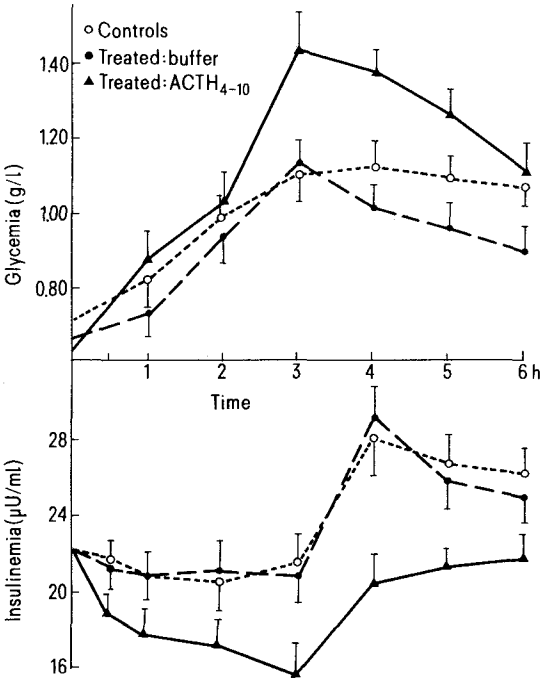

Figure 1. Effects of i.v. administrations of 130 µg/kg of $ACTH_{4-10}$ on plasma glucose and circulating insulin levels. The determinations were made during 6 h. Mean values with SE determined in 6 animals.

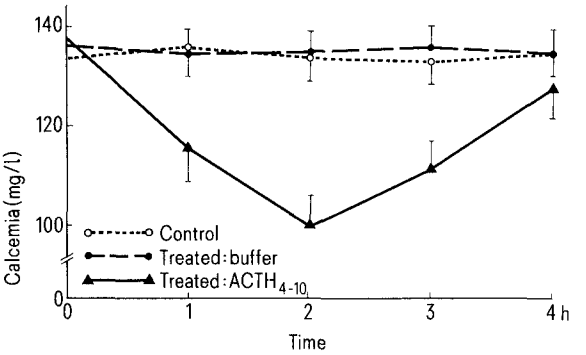

Figure 2. Effect of $ACTH_{4-10}$ (130 µg/kg in 0.2 ml phosphate buffer) on circulating calcium levels in rabbits. Data are given as means ± SE; n = 6.

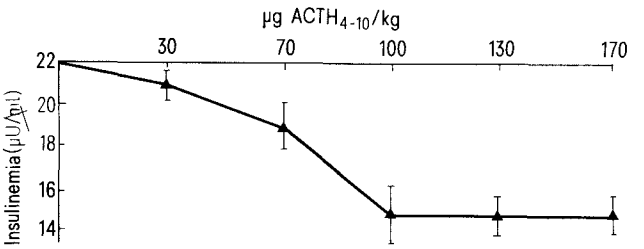

Figure 3. Dose response curve of insulinemia after i.v. administration of various amounts of $ACTH_{4-10}$. Data are given as means ± SE; n = 6.

$ACTH_{4-10}$ 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-