cuticle can definitely not be considered a direct effect of the active substance on the epidermal cells because of its similarity to untreated pharate next stage larvae shortly before ecdysis. The new cuticle is apparently stretched directly after ecdysis but before sclerotisation. Because treated animals cannot escape from the old cuticle, sclerotisation of the unstretched cuticle takes place underneath the old one.

In order to determine a possibly existing sensitive phase of this effect, we kept first instar larvae for various periods of time on treated filter paper. We could demonstrate that the larvae have to be in contact with the treated paper for at least 6 succeeding days to get a pronounced anti-ecdysis effect. No clearly defined sensitive phase could be detected in trials, where we kept first instar larvae on treated filter paper between the 3rd and 5th, 5th and 7th, 4th and 6th and between the 3rd and 6th day. 1 or 2 topical applications of 10 µg JH/larvae revealed, however, an increased sensitivity at the 3rd and 4th day of the first instar.

The anti-ecdysis effect could be demonstrated in all larval instars. In the last instar, however, permanent exposure to 10 µg JHA or 100 µg JH/cm² elicited ecdysis inhibition only in about 50% of the larvae. In this case, the anti-ecdysis effect was obviously overridden by the morphogenetic effect signalled by the formation of extra-larvae and all sorts of adultoids. The morphogenetic effect remained strongly expressed up to a dose of 0.1 µg JHA or 1 µg JH/cm². All morphogenetically effected males or females were found to be permanently sterile.

In *Hyalophora cecropia*, the emergence difficulties of adultoids could be explained as the result of a suppression of muscle development (RIDDIFORD[4]). During the larva-larva moult, however, no particular organ reconstruction takes place and consequently, the dissection of larvae of the German cockroach dying in ecdysis did not reveal any particular changes of musculature. DAVEY[5] reported a very interesting case of ecdysis inhibition in last instar larvae of *Phocanema decipiens* (Nematoda) treated with JH. Stimulation of the release of a neurohumoral factor from the brain caused disruption of the function of the exuvial fluid. The larvae died encapsulated in the undigested old cuticle. The moulting difficulties of *Blattella* larvae look very similar. We believe that the inhibition of metamorphosis and ecdysis are two separate effects of JH and its analogues. The ecdysis inhibition is the general effect of JH and can act in any stage of the postembryonic development of roaches. Much lower dosages are required to inhibit metamorphosis. Further experiments are now on the way in this laboratory to understand the mechanism of ecdysis inhibition and its relation to the morphogenetic effect of JH active compounds in both Hetero- and Holometabola.

Zusammenfassung. Das Juvenilhormon von *Cecropia* und eines seiner Analoga bewirken letale Häutungsstörungen in allen Larvenstadien von *Blattella germanica*.

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Urinary Kallikrein from Normal and Hypertensive Rats

According to several reports, there is a significant decrease in kallikrein activity in the urine of hypertensive rats[1,2] and of essential hypertensive patients, as compared with normals[3–5]. Furthermore, evidence has been provided that urinary kallikrein is similar to that contained in renal tissue[6] and that the excretion of this enzyme is correlated with sodium excretion[7,8]. It has been established that a good correlation exists between the esterase, kininogenase and oxytocic activities of the urine of normal rats[9], but no attempts have been undertaken to investigate the enzymatic kinetics of kallikrein obtained from the urine of hypertensive rats. On the other hand, it has not been ruled out that the occurence of an inhibitor could account for the low kallikrein activity.

In the present report, a comparative chemical study of the effect of inhibitors was undertaken between purified kallikrein obtained from the urine of normal and hypertensive rats.

Material and methods. Adult Sprague-Dawley rats were used. Two batches of urine were obtained from hypertensive rats: a) 1 of 860 ml from 17 rats (mean blood pressure, 178 mm Hg); and b) 1 of 820 ml from 11 rats (mean blood pressure, 152 mm Hg). A 3rd batch of 1,450 ml of normal rat urine was used as control. The operation to induce hypertension was performed according to GROLLMAN's procedure[10]. Blood pressure was measured at weekly intervals by the FRIEDMAN and FREED method[11]. For the experiment, only those rats showing a blood pressure consistently over 145 mm Hg were selected. The rats were placed in metabolic cages to collect urine in conditions which prevent contact with feces.

Purification step	KU/mg protein/min		Purification factor	
	Normal	Hypertensive	Normal	Hypertensive
1 Sephadex G-200	4.35	0.14	1	1
2 Sephadex G-200	6.25	0.22	1.48	1.57
CM-52	130.00	6.25	30.00	44.50

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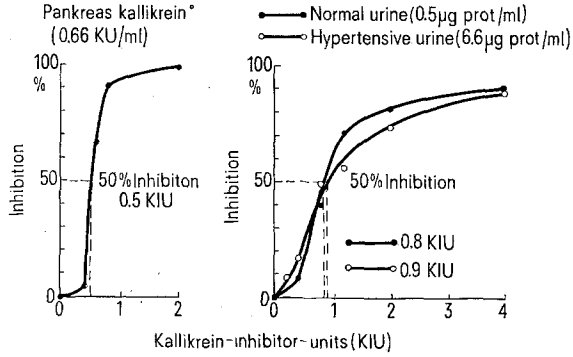

Fig. 1. Left side: Inhibitory effects of apronitin upon the esterase activity of bovine pancreas kallikrein (Padutin 0.66 KU/ml); Right side: ●---● kallikrein from normal rat urine (0.5 µg prot/ml); ○---○ kallikrein from hypertensive rat urine (6.6 µg prot/ml). Abscissa: amount (in KIU) of apronitin used. 50% of inhibition is shown by the broken lines (---)

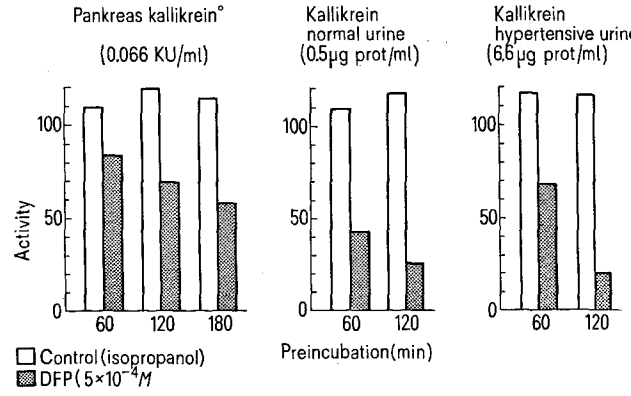

Fig. 2. Inhibitory effect of DFP upon the esterase activity of pancreas kallikrein (Padutin) and upon kallikrein obtained from normal and hypertensive rat urine respectively. Abscissa: % of the remaining esterase activity; ordinate: activity found at 60, 120 and 180 min after the addition of DFP or the solvent alone. BAEE (5×10^{-4} *M*), pH 8.0, Temperature 20–22 °C.

The 3 batches of urine were separately submitted to the first 5 steps of the purification procedure described by PORCELLI and CROXATTO[12]. The active fractions obtained from the last chromatographic step (carboxymethyl-cellulose column (CM-52))[12] were pooled, dialyzed against distilled water and then liophylized; finally the residue was dissolved in a 0.9% NaCl solution and stored at 20 °C until chemical and biological assays were performed.

The esterase activity of these purified enzymes, and of the corresponding peaks obtained in each of the chromatografic steps, were determined, using as substrate: benzoyl-L-arginine-ethyl ester (BAEE) at a final concentration of 5×10^{-4} *M*, according to the method of HESTRIN[13] modified by THRAUSCHOLD and WERLE[14]. Measurements were carried out at 20–30 °C in a spectrophotometer (Beckman DU) at 235 nm. Padutin (Bayer) was used as standard kallikrein, and the activity expressed in KU was calculated fron an experimental curve which was linear between 0.1 and 0.4 KU under the conditions described.

The effect of kallikrein inhibitors: apronitin (Trasylol, Bayer) and di-isopropylfluor-phosphate (DFP) was investigated in each purified sample. 0.8 KIU of apronitin dissolved in 2.5 ml triethanolamine buffer was introduced to kallikrein solution prior to the addition of BAEE (0.5×10^{-4} *M*).

To study the effects of DFP, a solution of this serine blocking agent in isopropanol (2×10^{-2} *M*) was used. It was added to the mixture of kallikrein and triethanolamine buffer, in order to get a final DFP concentration of 5×10^{-4} *M*. A similar mixture with an equivalent amount of isopropanol without DFP served as control. The esterase activity of these mixtures was studied at 60 and 120 min after the addition of either DFP or its solvent. At the end of each period, 2.5 ml of each solution was introduced into a spectrophotometer cuvette which contained the substrate BAEE.

Results and discussion. The esterase activity in the purified enzyme from normal urine reached up to 130 KU per mg and in the hypertensive rat urine it was only 6.25 KU per mg (Table). Notwithstanding this quantitative difference, a perfect correlation was found between their esterase and biological activities. Furthermore, the same constant ratio between esterase activity and oxytocic effect was also obtained in the lesser purified samples. It was also evident that the fact that the amount of inert protein in the hypertensive rat urine was 8–12-fold higher than normal, renders purification more difficult, and reduces the magnitude of the purification factor.

Results obtained by studying the kinetics and the effects of the inhibitors – apronitin and disopropylfluorophosphate (DFP) – further indicate that the differences between kallikreins of normal and hypertensive rat urine are only quantitative.

The Michaelis constant (KM), using BAAE as substrate, was similar for both samples. The KM for normal rat kallikrein was 1.1×10^{-4} *M*, and the KM for the hypertensive rat was 0.9×10^{-4} *M*. Their V_{max} was 300 and 8 KU/mg/min, respectively.

Kallikrein from both normal (0.5 µg) and hypertensive (6.6 µg) rat urine was inhibited by apronitin in a similar way; 50% of the inhibition occurred at the inhibitor's concentration of 0.8 and 0.9 KIU (Figure 1). In both cases the O.D. obtained (253 µU) was 0.120 in 10 min. In an equivalent amount of Padutin (0.066 KU/ml), a 50% inhibition was reached by the addition of 0.5 KIU of apronitin. These results show similar effects of apronitin upon equivalent enzymatic amounts of urinary kallikrein either from normal or hypertensive rats. Furthermore, it demonstrates that the apronitin has a higher inhibitory activity upon pancreatic kallikrein (Padutin) than on the rat urinary kallikrein.

DFP after 60 and 120 min of contact with the enzyme, either from normal or hypertensive rat urine, produced 5 and 75% of inhibition respectively. Under similar conditions Padutin was inhibited 16% and 31% after 60 and 120 min contact. These figures were calculated considering that isopropanol produced in all the mixtures a moderate but progressive increase of the esterase activity.

The similar effects of the inhibitors, the similar KM for esterase activity, and the same ratio between the oxytocic and esterase activities exhibited by both purified kallikreins from normal and hypertensive rat urine, give evidence that the much lower kallikrein activity found in the latter is due neither to the presence of an inhibitor, nor to a chemical difference with normal

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urinary kallikrein, but to a decreased excretion or production of this enzyme by the kidneys of the hypertensive rats.

Résumé. L'amoindrissement de l'activité kallikréinique urinaire chez les rats hypertendus (technique de GROLLMAN[10]) est due à une diminution de l'élimination de cet enzyme, puisque la cinétique enzymatique, l'action des inhibiteurs sur l'activité estérasique (sur BAEE) et oxytoxique est égale à celle trouvée chez la kallikréine urinaire des rats normaux.

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Effect of Prostaglandin E_1 on Cholesterol Biosynthesis in Rat Liver

In 1952 it was first reported that the ingestion of synthetic diets containing a large proportion of vegetable fat resulted in a reduction in the level of plasma cholesterol[1]. Subsequent studies demonstrated that the isocaloric substitution of an unsaturated fat diet for a saturated fat resulted in a decline of plasma cholesterol concentration[2-3].

The mechanism whereby unsaturated fat feeding lowers plasma cholesterol is far from being clarified. Since the liver is regarded as the major source of circulating cholesterol[4-5], the reduction in plasma cholesterol under these dietary conditions may be due to a decrease in the cholesterogenic acticity of the liver induced either by the polyunsaturated fatty acids or by some of their derivatives. Polyunsaturated fatty acids are precursors of prostaglandins[6]. Several mammalian tissues have been found to be capable of converting polyunsaturated fatty acids into prostaglandins[7-8].

It has also been postulated by KUNZE and VOGT[9] that the rate of prostaglandins formation within a tissue is determined by the availability of the substrate for the PGE synthetase. Several lines of evidence indicate that increased release of prostaglandins in tissues occurs after i.v. administration of polienoic fatty acids[10] and phoshолipase A[11].

Furthermore, in presence of essential fatty acids deficiency, there is a defect in the synthesis of prostaglandins[12]. This close association between polienoic fatty acids and prostaglandins suggested us a possible mechanism of action of essential fatty acids on cholesterol biosynthesis. If it could be demonstrated that prostaglandins have an inhibitory effect on hepatic cholesterogenesis, the decline in plasma cholesterol which follows the ingestion of polyunsaturated diet could be explained in terms of an increase formation of PGE_1 within the liver.

In order to shed some light on this hypothesis we studied the in vitro effect of PGE_1 on cholesterol synthetic activity of rat liver slices.

Materials and methods. Male Wistar rats (200–250 g) were used in this study. Liver slices were incubated in Krebs bicarbonate buffer in presence of ^{14}C-acetate and various amounts of PGE_1 (Upjohn, Company, Kalamazoo, Michigan, 49001). The rate of incorporation of labelled acetate into cholesterol (as digitonin precipitable sterols) fatty acids and metabolic CO_2 were assayed as described in a previous report[13].

Results and discussion. The in vitro effect of PGE_1 on the rate of incorporation of labelled acetate into fatty acids, cholesterol and CO_2 is illustrated in Figure 1. The addition of minute amounts of PGE_1 to the incubation system resulted in a stimulation of both cholesterogenesis and fatty acid synthesis. As the concentration of PGE_1 was increased we observed an inhibition of cholesterol and fatty acids synthesis as well as a significant reduction in the production of radioactive CO_2. PGE_1 therefore appears to have two opposite effects on the incorporation of acetate by liver slices, which is dependent on its concentration in the liver tissue. As both fatty acids and sterol synthesis, as well as CO_2 production, are equally affected by PGE_1, it may be speculated that PGE_1 interferes with the formation of active acetyl, the precursor for the biosynthesis of fatty acids and sterols.

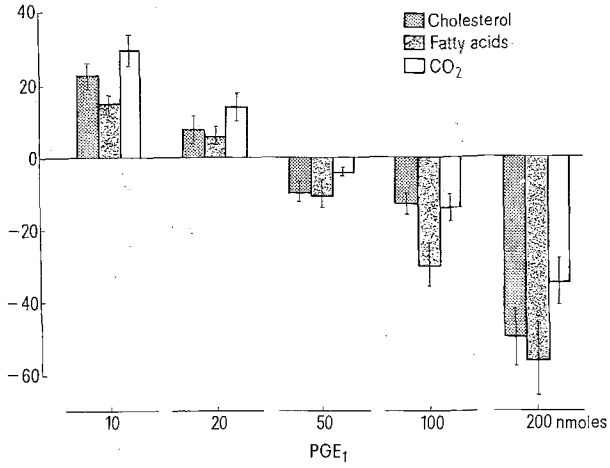

Fig. 1. The effect of increasing amounts of PGE_1 on the incorporation of acetate-2-C^{14} into cholesterol (as digitonin precipitable sterols) fatty acids and metabolic CO_2. Each flask contained liver slices (0.5 mm thick) and 5 ml of Krebs' bicarbonate buffer (pH 7.4), 25 μmoles of sodium acetate -2-C^{14}(specific activity 0.05 μCi/μmole).

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