arginine ethyl ester (BAEE) by kallikrein. The list includes amidines and indole derivatives, most of which have previously been shown to inhibit trypsin[13–15], and the series is rounded off by 2 strong inhibitors of chymotrypsin, α-ketoisocaproic acid[16] and *p*-aminophenylpyruvic acid[17]. The most effective inhibitors of kallikrein were among the amidines, and the ranking of these compounds as to potency was the same as described earlier for trypsin with the only notable exception that 4,4′-diamidinodiphenylamine moved into first place ahead of *p*-amidinophenylpyruvic acid. 3-Indolepyruvic acid turned out to be a stronger inhibitor of kallikrein than tryptamine, while the converse holds true for trypsin. Benzamidine, one of the compounds investigated here, has previously been studied by MARES-GUIA and DINIZ[5] for its influence on rat urinary kallikrein and the inhibition constant was reported as $1.15 \times 10^{-4} M$. Using the same substrate as those investigators, i.e., BANA, the K_i value of benzamidine for hog pancreatic kallikrein was determined as $6 \times 10^{-4} M$, the value of *p*-aminobenzamidine as $2.4 \times 10^{-4} M$, and the value of 4,4′-diamidinodiphenylamine as $1.5 \times 10^{-5} M$. The type of inhibition was competitive for all compounds.

The results in Table II confirm the findings of ROCHA E SILVA[9] that tyrosyl and methionyl bonds are hydrolyzed by commercial kallikrein. In addition, it is evident that esters of L-tryptophan, L-phenylalanine and L-leucine were readily split, and especially so when the alpha amino group was acylated. A comparison of the 2 kallikrein preparations reveals that the material in the first column had a much higher activity against non-arginyl esters than the one in the second column. However, the ratios of corresponding non-arginyl esterase activities in the 2 preparations were remarkably constant. This observation suggests that a single enzyme might have been responsible for all the chymotrypsin-like activities. Contamination by chymotrypsin itself could be ruled out by the absence of any hydrolysis of DL-norleucine ethyl ester, a substrate which is split by chymotrypsin almost as rapidly as the kallikrein substrate L-leucine methyl ester. In view of the recent discovery of a number of different kallikreins in hog pancreas[18], it has to be considered whether one or several of these enzymes may not be endowed with the ability to split also non-arginyl bonds. The mode of purification of kallikrein might favor the presence of one form over the other in different enzyme preparations, thus accounting for the variations in the ratios between BAEE-ase activity and non-arginyl esterase activity. The suggestion that native kallikrein or a modified form may possess the ability to split non-arginyl bonds is supported by the finding that 4,4′-diamidinodiphenylamine strongly blocks not only the hydrolysis of BAEE, but of all the other esters as well, that L-tyrosine ethyl ester (TEE) inhibits competitively the hydrolysis of BANA, and that, on the other hand, BANA impedes the hydrolysis of TEE[19].

Zusammenfassung. Es wird in 4,4′-Diamidinodiphenylamin der bis jetzt stärkste niedermolekulare Hemmstoff für Kallikrein aus Schweinepankreas gefunden, mit der Fähigkeit, Tryptophan-, Phenylalanin- und Leucin-Ester zu spalten.

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Occurrence of Rhodoquinone-9 in the Muscle of *Ascaris lumbricoides var. suis*

Rhodoquinone was first isolated by GLOVER and THRELFALL[1] from *Rhodospirillum rubrum*. Further work by MOORE and FOLKERS[2] has shown the true structure of rhodoquinone, in which one methoxyl group of ubiquinone-10 is replaced by amino group. Recently POWLS and HEMMING[3] isolated rhodoquinone-9 from *Euglena gracilis*. The occurrence of rhodoquinone in nature has hitherto been confined to only these few microorganisms in contrast to the ubiquitous existence of ubiquinone in the living systems. During the survey study on ubiquinone in parasitic nematodes, the authors have obtained the evidence that the mitochondrial fraction of *Ascaris* muscle contained a rhodoquinone analogue in the place of ubiquinone[4]. This was the first evidence showing the occurrence of rhodoquinone in an animal tissue. In order to characterize rhodoquinone from *Ascaris* muscle, further examination with a large amount of *Ascaris* muscle was carried out. Now the quinone has been isolated in a crystalline form and the spectroscopic examinations revealed that it must be rhodoquinone-9 just like that from *Euglena gracilis*.

Methods and results. Adult round worms, *Ascaris lumbricoides* var. *suis*, were collected freshly from the slaughter house. Worms were cut open longitudinally and freed from intestine, eggs, etc. The muscular layer was then scraped from the cuticle, and washed several times with 0.9% NaCl solution. Muscle strips thus obtained were stored at −15 °C. Homogenized in Waring blender the frozen materials were extracted with 10 volumes of ethanol-ether (3:1, v/v) for about 15 h. The combined ethanol-ether extracts were evaporated under reduced pressure over a warm bath. When the volume of the extract was reduced to 1/10 the original volume, aqueous ethanol suspension was extracted with *n*-hexane.

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The hexane extract was evaporated to dryness under N_2 gas.

The residue was applied on a column of silica-gel, eluted with hexane to remove lipid fraction, and then with hexane-benzene (1:1) to recover the violet quinone band. The quinone fraction was then purified by preparative thin-layer chromatography using Silica-gel G plates. The development with chloroform formed 2 purple bands. The upper band, though it has not yet been obtained in a pure form due to its instability, shows nearly the same UV-absorption as the lower and is assumed to be a precursor of the latter from the fact that the former easily forms the latter by repetition of thin-layer chromatography or other treatment. The lower band was extracted with ether and the residue was further purified by the repetition of preparative thin-layer chromatography using a mixture of hexane-chloroform as the solvent, and finally recrystallization from cold methanol to deep violet crystals of m.p. 66.5–67° (5 mg from 1600 g of the muscle), M^+ 779.618 *m/e* (Calcd. for $C_{53}H_{81}O_3$ N, 779.622), λ_{max}^{EtOH} 285, 515 nm, ν_{KBr} 3470, 3330, 2850–3050, 1643, 1600 cm^{-1}.

Synthetic rhodoquinone, m.p. 38–42.5°, was prepared from ubiquinone-9 by ammonolysis[2,3] followed by the separation by preparative thin-layer chromatography. The natural quinone and the synthetic sample show entirely the same UV- and IR-absorptions, identical fragmentations in mass spectra, and identical Rf values in thin-layer and reversed phase thin-layer chromatography[5].

Discussion. Ubiquinone is the most widely distributed benzoquinone in nature. Its distribution is closely correlated with the aerobic metabolism of a tissue or organism, a pattern which is consistent with the evidence that ubiquinone is a coenzyme in the electron transport system in mitochondria[6]. Although the time course study with labelled *p*-hydroxybenzoate has shown that rhodoquinone is a product of ubiquinone metabolism[7], the occurrence of rhodoquinone has been confined to only few microorganisms. Precise physiological role of rhodoquinone has not yet been determined. While in the microorganisms, *R. rubrum* and *E. gracilis*, both ubiquinone and rhodoquinone are detectable, in *Ascaris* worm rhodoquinone exclusively. Preliminary studies on the intracellular distribution of rhodoquinone-9 in the *Ascaris* muscle provided the evidence that rhodoquinone in the mitochondrial fraction, in which could be observed many mitochondria poor in cristae by the electron microscope, accounted for about 65% of the total in the muscle homogenate. These facts suggest that rhodoquinone may play some physiological role on the mitochondrial function in the *Ascaris* worm. Precise physiological role of this rhodoquinone-9 in the *Ascaris* worm remains to be evaluated.

Zusammenfassung. Es gelang, aus der Epithelmuskelzelle von *Ascaris lumbricoides* var. *suis* Rhodochinon (Methoxylgruppe in Ubichinon mit einer Aminogruppe substituiert) zu extrahieren und kristallin zu gewinnen. Die Spektralmessungen (UV-, IR-, Massen-Spektren) ergeben, dass es sich beim Rhodochinon um Rhodochinon-9 handelt.

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Selenium Toxicity: Effect of Fluoride

Results from epidemiological studies among children and from animal experiments indicate that consumption of small amounts of selenium during the period of tooth development increases the suceptibility to dental caries[1]. However, the effect of fluoride, which is a well-known agent for prevention of caries, on selenium metabolism has received only scant attention. MOXON and DUBOIS[2] reported that the combined administration of fluoride and selenium to rats increased the toxic action of selenium. The present study was undertaken to provide further data on the effect of fluoride on selenium toxicity.

30 male, weanling rats of the Sprague-Dawley strain were equally divided into 2 groups and housed in individual cages with raised screen bottoms. The first group received drinking water containing 3 ppm of selenium as sodium selenite and 50 ppm of fluoride as sodium fluoride. The second group drank water having only 3 ppm of selenium.

Both groups of animals were fed a diet commonly used in experimental caries research having the following composition (per cent): ground corn, 64; powdered whole milk, 30; alfalfa meal, 3; irradiated yeast, 2; sodium chloride, 1. Food and water were provided ad libitum and the consumption measured accurately by methods described in previous works[3,4]. The intake of water was measured daily but that of food only on 3 consecutive days during each of the 4 weeks of the experimental period.

The results are presented in the Table and indicate that the combined administration of selenium and fluoride to rats did not increase the severity of symptoms characteristic of chronic selenosis compared with the

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