Contraction of Frog Stomach Muscle and Frog Heart in Electrolyte-Free Solutions

Frog stomach muscle when frequently washed with half-isotonic (0.112 *M*) solution of sucrose, and frog heart perfused with similar solution lose nearly all sodium in 1 h[1,2], but exhibit spontaneous contractions[3-6], with conducted action potentials for several hours[7-15]. They lose about 90% of their sodium in the first 15 min, and in 1 h the sodium content of both becomes less than 0.05 m*M*/kg of wet muscle[2]. There is however a striking difference between the mechanical performance of the stomach muscle and that of the heart. The stomach muscle shows spontaneous contractions at all temperatures (experimental range 15–35 °C) for about 24 h. The frog heart beats for about 24 h at 5 °C, for about 2 h at 25 °C[16], and at high temperatures (30–35 °C) it passes into contracture and does not beat at all.

There is a remarkable difference between the behaviour of adenosinetriphosphate (ATP) in sucrose-soaked frog stomach muscle and in frog heart. The ATP content of frog stomach muscle does not differ in any way whether the muscle is soaked in saline or in sucrose solution[17]. But in frog heart both ATP and creatine phosphate fall in sucrose solution, more at 25 °C than at 5 °C, and the fall correlates with the decline in the force of contraction[16].

These experiments therefore suggest that sodium in muscle has an important metabolic function of preserving the ATP and failure of function in sodium-free solutions is most likely due to loss of ATP rather than to any direct action on the excitability mechanism.

Zusammenfassung. Der Verlust der Reizbarkeit bei Froschherz- und Magenmuskulatur auf verschiedenen Temperaturstufen verläuft parallel mit einem Adenosintriphosphat-Verlust, was andeutet, dass das Nichtfunktionieren in natriumfreier Lösung von einem Adenosintriphosphat-Verlust abhängig ist.

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3,4-Dihydroxycinnamic Acid, an Antithiamine Factor of Fern

According to our previous publications, fern (*Pteridium aquilinum*) contains antithiamine substances[1,2]. These early experiments have shown that the active agent was a thermostable, water-soluble, small molecule, which moved in an electric field to the anode[3,4].

Later a good purification of the antithiamine agent was achieved, although the substance could not be isolated in pure form. This antithiamine factor, called Hydrolysate II, was a red-brown amorphous substance, free of N, P, S and halogenes[5]. The specific antithiamine activity of this factor was confirmed by the experiments with the single nerve fibre of the frog by VON MURALT and PETROPOULOS[6] and PETROPOULOS[7].

These experiments have recently been resumed. The antithiamine activity was determined by the thiochrome and microbiological methods. The purification process, which has been substancially altered, is summarized in Figure 1. The antithiamine activity of the dark-brown, oily ethyl acetate phase increased, while the activity of the water phase remained unchanged. The most active fraction of the column chromatography (I) was rechromatographed.

The purification effect of the column chromatography has been established by polyamid thinlayer chromatograms. Seventeen components could be separated from the ethyl acetate phase (see also Figure 2). One of the components (shaded spot) possessed the highest antithiamine activity (Rf = 0.47). This could be isolated as a uniform substance.

This compound is a microcrystalline substance with a melting point 190–192 °C. Its antithiamine activity is very high, namely 1800 µg/mg, according to the thiochrome method (time effect curve) and 2300 µg/mg determined microbiologically. The activity is pH and temperature-dependent, being higher in slightly alkaline solutions (pH 7.8), than at pH 6.0.

The substance is thermostable; the antithiamine activity remained unchanged after boiling 2 h by reflux. It is soluble in water and ether and easily soluble in ethanol,

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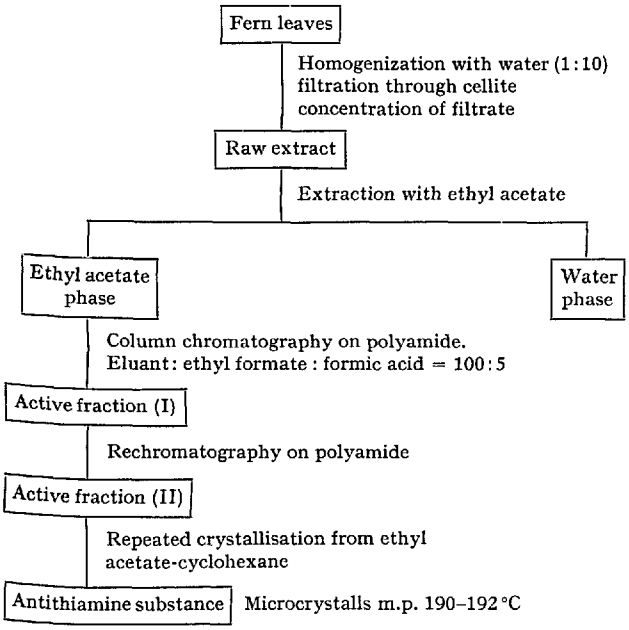

Fig. 1. Scheme of the isolation of a thermostable antithiamine factor of fern.

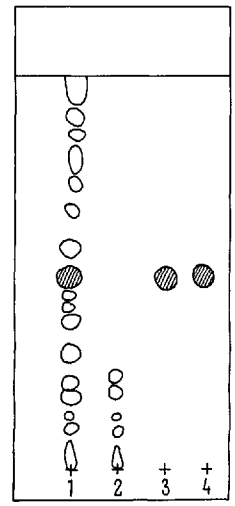

Fig. 2. Thin-layer chromatogram of different stages of the purification. (1) Ethyl acetate phase, (2) water phase, (3) isolated antithiamine substance, (4) 3,4-dihydroxycinnamic acid. Adsorbent: polyamide; developing solvent: ethyl formate: formic acid = 100:5; spray reagent: 1% diphenylboric acid β-amino ethyl ester in methanol.

acetone and ethyl acetate. The microanalyses gave the following results: C, 60.83%; H, 4.57%; O, 33.8%. The molecular weight determined by mass spectrography is 180.

The isolated antithiamine compound of fern was identified as 3,4-dihydroxycinnamic acid by the great agreement of the UV, IR and NMR-spectra, the identical behaviour in thinlayer chromatography (Figure 2), as well as of the other characteristic properties (MP, MW, results of the microanalyses).

The caffeic acid in fern seems to be only partly in free form; another part arises during the extraction (at pH 6.0) from a precursor not yet isolated.

In this connection it is interesting to mention that according to recent experiments (SOMOGYI and BÖNICKE[8]) different *o*-diphenols possess a marked antithiamine activity.

Further investigations to characterise the precursor and to elucidate the mechanism of the antithiamine effect of 3,4-dihydroxycinnamic acid are in progress[9].

Zusammenfassung. Die Isolierung eines Stoffes mit Antithiaminwirkung aus Farnkraut und seine Identifizierung mit 3,4-Dihydroxyzimtsäure wird beschrieben.

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Utilization of D-Tyrosine by Vertebrate Skin Tyrosinase

During the course of an evolutionary study of vertebrate melanogenesis based upon anatomic and subcellular tyrosinase distribution and activity[1], skin enzyme preparations from several vertebrates were found to utilize D-tyrosine. Skin enzyme preparations were incubated as previously described[2]. When uniformly labeled L-tyrosine-^{14}C or DL-tyrosine-2-^{14}C were used as substrate, the net tyrosinase activity obtained from the latter substrate was higher than that obtained from the former substrate, although the concentration and specific activity of L-tyrosine-^{14}C as well as the enzyme preparations were the same in both series of experiments. As D-tyrosine-^{14}C was not available, the D-tyrosine utilization was determined indirectly by use of L-tyrosine-1-^{14}C, uniformly labeled

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