gration. This latter preparation is completely insensitive to atractyloside (Low, Vallin & Alm, 1963).

Further investigations are needed to explain this difference.

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Inhibition by quercetin of magnesium-, sodium- and potassium-activated adenosine triphosphatase

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The flavonoid quercetin increases the strength of the heart beat, an effect which is not due to a local release of catecholamines or to a direct interaction with beta-receptors (see Santi, 1966). The potentiation of the effect of quercetin by decrease in calcium concentration and the observation (Carpenedo, Ferrari & Santi, 1968) that the drug promotes the release of bound calcium from isolated guinea-pig atria, suggests that quercetin inteferes with the mechanisms regulating the movement and utilization of calcium ions. We have studied the effect of quercetin on magnesium, potassium and sodium-activated adenosine triphosphatase.

The enzyme was prepared from beef heart (Matsui & Schwartz, 1966) and used after the first sodium deoxycholate treatment. At this level of purity the ouabain-sensitive portion of the freshly prepared enzyme was 50–55% of the total in the presence of 100 mM sodium and 20 mM potassium. The specific activity (ouabain-sensitive plus ouabain-insensitive) was 20–22 μ-moles adenosine triphosphate split/mg protein per hr at 37° C. Quercetin in concentrations of 0.001–0.2 mM was an active inhibitor of the enzyme. 50% inhibition was reached with 0.04 mM quercetin at level of 50 μg enzyme protein. Salient features of the inhibition were: (*a*) the effect was dependent on the ratio inhibitor/enzyme concentrations rather than on the concentration of the inhibitor in the incubation medium ; (*b*) unlike ouabain but like oligomycin, quercetin produced a significant inhibition of the magnesium-stimulated adenosine triphosphatase. Mitochondrial 2-4-dinitrophenol or magnesium-stimulated adenosine triphosphatase was also affected by quercetin ; (*c*) the inhibitory effect was more manifest at high (300–500 mM) sodium concentration or low (1 mM) potassium concentration.

These results support the possibility that quercetin influences the transport of ions across the cell membrane. Moreover, they suggest that quercetin inhibits the magnesium-, sodium- and potassium-activated adenosine triphosphatase by a different mechanism from that of cardiac glycosides.

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A possible reaction mechanism for the enzyme histaminase

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Pig plasma histaminase is a copper-pyridoxal-phosphate enzyme (Buffoni & Blaschko, 1964; Blaschko & Buffoni, 1965; Buffoni, 1966) which catalyses the general reaction:

$$R\text{CH}_2\text{NH}_2 + \text{O}_2 + \text{H}_2\text{O} \longrightarrow R\text{COH} + \text{NH}_3 + \text{H}_2\text{O}_2$$

Direct evidence for the formation of a Schiff base between enzyme and substrate in the first step of reaction has been obtained using ^{14}C-histamine. Under anaerobic conditions the enzyme binds 3 moles of histamine per mole of protein, forming an imine bond. The borohydride-reduced Schiff base, pyridoxyl-histamine-5′-phosphate has been isolated and identified by paper and thin-layer chromatography and by fluorescence properties (Buffoni, 1966).

Direct evidence that water is not involved in the ammonia formation has been obtained by using $^3\text{H}_2\text{O}$ and isolating the ammonia formed in the reaction (Buffoni & Della Corte, 1967).

Electron spin resonance (ESR) experiments have shown that the copper remains divalent in the oxidized and reduced enzyme (Buffoni, Della Corte & Knowles, 1968) although there were changes in ESR line shape after reduction and of the ESR signal throughout the catalytical cycle (quench flow method).

These and other experiments on the kinetics of the reaction suggest a mechanism of reaction for the enzyme histaminase in which the Schiff base formation represents the rate limiting step.

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Immunosuppressive activity of methotrexate and arabinosyl cytosine in mice bearing L1210 leukaemia

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We have studied the inter-relationship between chemotherapy and immunity in a tumour-host system in which, in addition to the possible tumour-specific antigenicity,