Purification and Properties of a Fraction of Chicken Pepsin

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By means of modifications of the methods of Bohak (1969) and Donta & Van Vunakis (1970), evidence has been obtained for the presence of at least five chicken pepsinogens in a mixed batch of chicken proventriculae. One pepsinogen has been isolated in pure form by repeated chromatography of a slightly purified extract on DEAE-cellulose columns followed by gel filtration on a column of Sephadex G-100. Agarose-gel electrophoresis indicated the presence of one protein component with potential proteolytic activity.

The pure pepsinogen could be activated at either pH 2.0 or 4.0 to the same single pepsin. The pepsin could be assayed by means of milk clotting at pH 6.2, haemoglobin proteolysis at pH 1.8 or hydrolysis of phenyl sulphite at pH 3.6. In the bis-phenyl sulphite assay the production of phenol is followed spectrophotometrically (Stein *et al.*, 1971); chicken pepsin reacted with the substrate at approximately the same rate as did swine pepsin.

Gel-filtration experiments indicated that pepsinogen and pepsin had apparent molecular weights close to 43 000 and 42 000 respectively. The pepsin appeared to exist as a dimer under certain conditions at pH 4.6 and below, as a monomer-dimer equilibrium mixture at pH 6.0 and as a monomer at pH 7.0.

The pepsin reacted rapidly with *p*-chloromercuribenzoate, at both pH 7.0 and 4.6, to the extent of about 0.8 mol/mol of pepsin, thus indicating the presence of 1 mol of thiol group/mol. This modification did not affect the enzymic activity. The pepsinogen reacted slowly with the same reagent to the extent of about 1.3 mol/mol of pepsinogen. The modified product could be activated normally and the derived pepsin still contained the mercuribenzoate moiety, thus indicating that the same thiol group was reacting in both the pepsin and the pepsinogen.

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Formation and Utilization of Ketone Bodies by Tissues of the Adult Pigeon

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Although mammalian ketone-body metabolism has been much investigated (for review see Williamson & Hems, 1970), little is known of the occurrence or metabolism of these compounds in other forms of life. However, Bailey *et al.* (1971) have reported the occurrence of ketone bodies in the blood of adult pigeons (*Columba livia*) and haemolymph of adult locusts (*Schistocerca gregaria*). In the present study the production and utilization of ketone bodies by various tissues of adult pigeons and the effect of starvation on these processes have been studied.

Acetoacetate and D-3-hydroxybutyrate are found in the blood of adult pigeons, the total ketone-body concentration in the blood of male birds being 0.14 mM with a D-3-hydroxybutyrate/acetoacetate concentration ratio of 7, whereas in female birds the total concentration of ketone bodies was 0.14 mM with a D-3-hydroxybutyrate/acetoacetate concentration ratio of 3. Although in both sexes starvation increased the blood concentration of both ketone bodies, that of D-3-hydroxybutyrate increased to a greater extent, leading to an approximately threefold increase in the D-3-hydroxybutyrate/acetoacetate concentration ratio in each sex. The major site of ketone-body production is the liver. Production of acetoacetate and D-3-hydroxybutyrate by liver slices is unaffected by the addition of potassium palmitate to the incubation medium, and the rate of ketone-body production is similar in liver slices from fed or starved birds. [3-¹⁴C]Acetoacetate was readily oxidized by preparations of heart, breast-muscle, kidney and brain but not by liver slices. However, only kidney slices from fed or starved pigeons and brain slices from starved pigeons would rapidly oxidize D-3-hydroxy[3-¹⁴C]butyrate. It appears that acetoacetate provides an important source of energy for all extrahepatic tissues during starvation, whereas D-3-hydroxybutyrate is probably only an important source of energy for the kidneys and brain. Low activities of D-3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) were found in all tissues except brain and kidney. Starvation caused a marked fall in the activity of D-3-hydroxybutyrate dehydrogenase in the kidney and a slight rise in activity in the brain.

The results suggest that ketone bodies play a similar role in the pigeon to that in mammalian systems, i.e. they are formed in the liver (which cannot oxidize them) during fatty acid oxidation and provide an important source of energy for the extrahepatic tissues (Krebs, 1961).

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The Relation between Calcium Ion Transport and Adenylate Cyclase Activity in Myocardial Sarcoplasmic-Reticulum Preparations

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The regulation of Ca^{2+} concentration in relation to myofibrils plays a central role in the production of contraction and relaxation in cardiac muscle (Langer, 1968). The calcium pump of the sarcoplasmic reticulum is a major factor producing relaxation by removing Ca^{2+} from the region of the myofibrils, and may secondarily produce changes in Ca^{2+} release on activation leading to alterations in myocardial contraction. Cyclic AMP (adenosine 3':5'-cyclic monophosphate) has been shown to stimulate the calcium pump (Epstein *et al.*, 1970; Shinebourne & White, 1970), and variations in the production of cyclic AMP by adenylate cyclase may form the basis of a regulating mechanism.

We have measured Ca^{2+} uptake (Gertz *et al.*, 1967) and adenylate cyclase activity (Krishna *et al.*, 1968) in the same preparations of fresh sarcoplasmic reticulum from canine myocardium. Ca^{2+} uptake was measured in the presence of oxalate and imidazole in duplicate at 1 min intervals, and the mean uptake over 5 min was used in the calculation. Under these con-

ditions Ca^{2+} is known to cross the membrane physically to storage sites inside the vesicle (Hasselbach & Makinose, 1963). The determinations were repeated in the presence of 1 mM-noradrenaline. Adenylate cyclase activity was enhanced by adding 10 mM-NaF and was calculated from the mean gradient of duplicate values at 5 and 10 min. The absence of mitochondrial contamination was confirmed by electron microscopy and by the absence of succinate dehydrogenase activity.

Noradrenaline was shown to increase adenylate cyclase activity significantly, even in the presence of F^- . The molar ratio of Ca^{2+} transported to cyclic AMP formed averaged 189 in controls and 257 in the presence of noradrenaline. On the basis of the adenylate cyclase activity and the time at which the rate of Ca^{2+} uptake reached equilibrium, the maximal density of β -adrenergic receptor sites in the sarcoplasmic-reticulum membrane was calculated to be $1/25000 \text{ sq. } \text{\AA}$. The enhancement of F^- stimulation of adenylate cyclase by noradrenaline and the independent increase in activity produced by glucagon (Entman *et al.*, 1969) suggest that adenylate cyclase activity in the sarcoplasmic-reticulum membrane can be regulated in several different ways.

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