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Influence of Biliary Constituents in an Acid Medium on the Micellar Solubilization of Unesterified Fatty Acids of Sheep Duodenal Digesta

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In sheep, the major lipid fraction of postruminal digesta comprises unesterified fatty acids (mainly stearic acid, palmitic acid and octadecenoic acid) (see Garton, 1967); these acids are associated with particulate matter (Lennox, Lough & Garton, 1965). Though Hofmann (1966) suggested that partial ionization of fatty acids is of importance for their micellar solubilization in the intestinal lumen, Lennox & Garton (1968) found that unesterified fatty acids were absorbed from the upper jejunum of the sheep where the luminal pH was in the range 2.8–5.0, indicating that un-ionized fatty acids were probably present in a soluble form. Accordingly the effect of biliary components on the solubilization of fatty acids under acid conditions was investigated with upper-duodenal digesta obtained from a cannulated grass-fed sheep.

Duodenal contents (8ml.) were shaken at 37° and at pH 4.0 (sodium hydrogen carbonate was added, if necessary) for periods of up to 24hr. with sheep gall-bladder bile (2ml.) or aqueous solutions (2ml.) of bile salt (as taurocholate) with and without bile lecithin or bile lysolecithin or both in amounts corresponding to those present in the whole bile which (per 100ml.) contained 7.8g. of bile salt, 885mg. of lecithin and 255mg. of lysolecithin. The amount and composition of the unesterified fatty acids were determined in the original digesta and in the micellar and solid phases after incubation.

With whole bile, maximal solubilization was reached within 90min. giving a net transfer of

5.6mg. of fatty acid to the micellar phase from 9.1mg. originally associated with particulate matter; a preferential solubilization of stearic acid was observed.

In relation to the amount of fatty acids solubilized in the presence of whole bile the proportion of fatty acid taken into solution when taurocholate alone was added to duodenal digesta was about 30% while the addition of lecithin increased this to 70%. Corresponding values for lysolecithin and for lecithin plus lysolecithin were 50 and 80% respectively. Under all these conditions, solubilization of fatty acids was non-selective though maximal solubilization was reached apparently no less rapidly than with whole bile.

This solubilization of un-ionized fatty acids resembles that of the insoluble non-swelling amphiphile cholesterol in a bile salt–lecithin–water milieu (Small, 1968).

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Electrophoretic Study of the Small Subunits of the Myosin Molecule

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On polyacrylamide-gel electrophoresis of myosin from adult rabbit white muscle in the presence of 8M-urea and 10mM-mercaptoethanol, four main bands were obtained. Often, even with very fresh myosin, two additional faint bands were apparent, but these appeared to be modifications of the major bands, for on storage further bands appeared, particularly at alkaline pH values and in the absence of thiol compounds. A considerable proportion of the applied sample remained at the origin when the gel was made up in 8M- or 12M-urea.

In myosin from rabbit heart muscle, the fastest moving band was completely absent. Myosin from rabbit red muscle gave a pattern intermediate between those obtained with white- and cardiac-muscle myosin, with the fastest band less dense than the slower ones. Locker & Hagyard (1968) have reported similar results using acetylated myosins. Myosin from foetal and 1-day-old rabbits gave a much more complex picture than that from adults. This was probably due to the difficulties in removing impurities from these preparations, but a band pattern similar to that of red muscle could be identified. Four-band patterns very similar to those

obtained with adult-rabbit white-muscle myosin were shown by myosin from skeletal muscle of the rat, mouse, pigeon and hen.

Subfragment 1 (Mueller & Perry, 1962) prepared from myosin by papain digestion (S. Lowey, personal communication) of rabbit white muscle yielded three bands in an 8M-urea gel. One of these had an electrophoretic mobility identical with that of the fast band obtained with adult myosin.

None of the bands obtained from whole sarco-plasm, actin, troponin and tropomyosin coincided with those from myosin studied under identical electrophoretic conditions.

Although it cannot be concluded finally from these studies that the low-molecular-weight components are integral parts of the myosin molecule, their consistent nature and the relation between them and different myosin types suggest that they are. It is suggested that myosin consists of isoenzymes that differ in the electrophoretic mobility of their small subunits and in the extent of methylation of the histidine in the main chains (Trayer, Harris & Perry, 1968). On this hypothesis, white-muscle myosin would contain more of the isoenzyme composed of electrophoretically fast small subunits than myosin isolated from red, foetal and cardiac muscles.

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The Sites of Action of Human and Swine Pepsins on the B Chain of Oxidized Insulin

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Five different pepsins have been found in swine (Ryle, 1965) and at least five in man (Etherington & Taylor, 1967, 1969). The major fundic enzymes, swine pepsin A and human pepsin 3, have analogous features, as have swine pepsin C and human pepsin 5 (Taylor, 1968). Functionally, swine pepsin C (Ryle & Porter, 1959) differs from swine pepsin A in that it can cleave only six of the ten bonds in the insulin B chain that are susceptible to pepsin A at pH 2.0 (Sanger & Tuppy, 1951) (see Fig. 1).

Human and swine pepsins sometimes display a second pH optimum against proteins above pH 3, and many synthetic substrates are hydrolysed maximally near pH 4 (see Taylor, 1968). We have, therefore, investigated the sites of action of purified swine and human pepsins at both pH 1.7 and pH 3.4.

Swine pepsin A was purified from a crystalline preparation, and swine pyloric pepsin, which was chromatographically similar to pepsin C, was obtained from a pyloric pouch secretion. Human pepsins 3 and 5 were separated from normal gastric juice.

At pH 1.7 human pepsin 3 failed to cleave bonds 1-2 and 23-24, but was otherwise similar to pepsin A. Swine pyloric pepsin differed from swine pepsin C by its ability to hydrolyse bonds 1-2 and 23-24; but hydrolysis at bond 11-12 was negligible. The specificity of human pepsin 5 was essentially similar to the specificity of swine pyloric pepsin except for its failure to attack bond 1-2.

Fig. 1. Cleavage by swine pepsin A and pepsin C of the oxidized B chain of insulin. CySO₃H, cysteic acid.