precipitated from liver and spleen is unknown. The values obtained with cortex and muscle probably represent the lowest sensitivity of the method with tissue extracts, as no specific binding of [6,7-³H]oestradiol-17 β to cortex was detected by gradient centrifugation; anterior hypothalamus, contained a 9S binding protein and, with it, more ³H radioactivity was precipitated by protamine (25%) than with cortex. After labelling of the 10⁵g supernatant from muscle with 1.0nM-[7 α -³H]-dehydroepiandrosterone sulphate or [7 α -³H]testosterone sulphate, 5% of the ³H radioactivity was precipitated by protamine, indicating the virtual absence of non-specific interaction between the polycation and steroid sulphates. After equilibration of 1.0nM-[6,7-³H]oestradiol-17 β with albumin, serum or phosphovitin, less than 5% of the ³H radioactivity was precipitated by protamine.

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Acetoacetate Formation in Bovine Tissues

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Studies with preparations *in vitro* have demonstrated that the liver and the epithelial lining of the rumen wall are important sites of ketogenesis in the healthy and the ketotic ruminant (see Hibbitt & Baird, 1967). In the cow it has also been claimed, on the basis of observations made in the intact animal, that the mammary gland may also be ketogenic, at least during ketosis (Kronfeld, 1963).

Krebs, Wallace, Hems & Freedland (1969) have shown, in rat liver, that measurement of ketone-body production from slices may not give a true indication of ketogenic potential. An alternative to the use of slices and tissue pieces is to assay the activity of the four enzymes involved in acetoacetate formation from acetyl-CoA (acetoacetyl-CoA thiolase, HMG-CoA* synthase, HMG-CoA lyase and acetoacetyl-CoA deacylase) in homogenates of the tissues concerned. In the present study these enzymes were assayed by the methods of Williamson, Bates & Krebs (1968) in homogenates of bovine liver, rumen epithelium, mammary gland and kidney.

HMG-CoA lyase was assayed in only liver and rumen epithelium from healthy cows. The other three enzymes were assayed in each of the four tissues derived from healthy lactating cows and

from cows suffering from spontaneous ketosis. No significant differences were found in the activities of these three enzymes in the ketotic tissues as compared with the healthy tissues.

The activities of the enzymes in bovine liver were similar to those reported for the corresponding enzymes in rat liver, indicating that in the cow, as in the rat, acetoacetate formation proceeds mainly via the HMG-CoA pathway.

Rumen epithelium was found to contain thiolase, HMG-CoA synthase and HMG-CoA lyase, but no deacylase. It is therefore clear that acetoacetate formation must take place exclusively by way of HMG-CoA in this tissue. This observation is of some interest and demonstrates that the HMG-CoA pathway for acetoacetate synthesis is not confined to the liver in ruminants, as it appears to be in non-ruminants.

Thiolase and deacylase were both present in bovine kidney tissue, but not HMG-CoA synthase. Neither HMG-CoA synthase nor acetoacetyl-CoA deacylase was present in bovine mammary gland, although there was some thiolase activity.

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Lipolytic Activity in the Pancreas

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The major lipolytic activity of the pancreas is attributed to the pancreatic lipase, but the organ also contains a relatively high content of lipoprotein lipase, which preferentially degrades the triglyceride moiety of lipoproteins, in contrast with pancreatic lipase, which acts on emulsified triglyceride. Although different reactivity to inhibitors has been reported for the two enzymes, LeVeen, Giordano & Zinberg (1961) and Kessler, Finkel, Dreiling & Janowitz (1963) have been unable to ascertain whether the lipoprotein lipase activity of the pancreas is a secondary property of the conventional lipase or is due to the presence of a separate enzyme.

In the present study the lipolytic activities of two different preparations from the pancreas have been measured by using as substrates a commercial triglyceride emulsion both in the free and albumin-activated state for determination of triglyceridase and lipoprotein lipase activity respectively.

Each preparation exhibited the properties of both enzymes, being inhibited by NaF and activated by

*Abbreviation: HMG-CoA, β -hydroxy- β -methylglutaryl-CoA.