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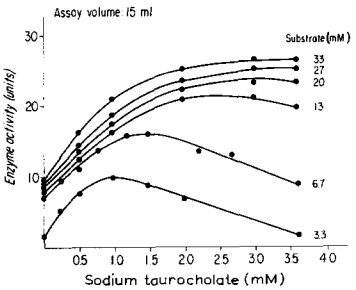
The influence of sodium taurocholate on the pancreatic lipase–substrate adsorption and activity

The purpose of these experiments was to determine the role of sodium taurocholate in the enzyme–substrate interaction and the enzymatic activity. In the past this has been the subject of much speculation. As early as 1932, GLICK AND KING[1] suggested that pancreatic lipase (glycerol-ester hydrolase, EC 3.1.1.3) activity was dependent on the surface area of the substrate. But it was not until 1965 that BENZONANA AND DESNUELLE[2] confirmed this by showing a quantitative relationship between the substrate surface and lipase activity. They thought that bile salts favored lipolysis by affecting enzyme adsorption to the substrate. WILLS[3] suggested that bile salts promote an alignment of enzyme molecules on the substrate surface. A review of other postulated functions of the bile salts on lipolytic activity has been presented by SCHOOR[4].

The method of purification and assay of porcine pancreatic lipase has been described by SCHOOR AND MELIUS[5]. The Sephadex G-200-treated water-soluble enzyme of that preparation was utilized. One unit of enzymatic activity was defined as that amount which would liberate 1 μequiv of fatty acid per min as determined by NaOH titration. The specific activity is expressed as units/mg protein. The adsorption experiments were carried out in the following manner. Assay solutions were prepared and brought to 0° 10 sec after the introduction of the enzyme. The solutions were then centrifuged in a Beckman Model L ultracentrifuge for 30 min at 100 000 $\times g$. The olive oil collected at the top of the tube, which was then pierced at the bottom in order to drain the clear liquid phase. Replicate assays were performed on this aqueous solution by substituting 10 ml of it for the 10 ml of water used in the normal assay. Since the olive oil emulsion contained gum arabic at a concentration of 10%, there was a consequent increase of gum arabic in the replicate assay mixture. This had no effect on the lipolytic activity.

The "adsorbed" and "unadsorbed" activities were defined as those found after centrifugation in the olive oil top part and the aqueous bottom part of the tube, respectively. In all experiments the enzyme protein used was equivalent to 26.0 units of activity. Since replicate assays were made on the aqueous phases only, their activities were subtracted from the initial 26.0 units and called "adsorbed" activity. All replicate assays were made under optimum conditions (5 ml substrate and 3.6 mM sodium taurocholate) regardless of the conditions under which the adsorption took place. In the cases where the sodium taurocholate concentration was lower than 3.6 mM in the replicate assay mixture, it was increased to that level. In the cases where it was higher, the activity obtained was adjusted according to Fig. 1. We used substrate concentration instead of substrate surface area making certain that all emulsions were prepared identically. We were thus dealing with a constant proportionality factor between the two.

Fig. 1 shows the correlation between substrate and sodium taurocholate concentration. While there is a maximum for each substrate concentration, there seems to be no simple relationship between these maxima and the amount of substrate. Fig. 2 indicates that the amount of sodium taurocholate does not significantly influence the



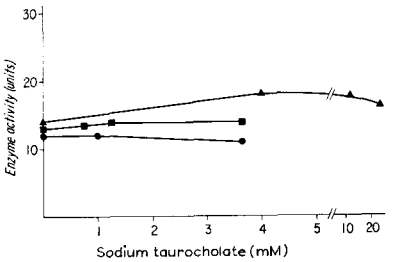


Fig. 1. Effect of sodium taurocholate on the lipolytic activity at various substrate concentrations. Total enzyme protein was 8.5 μg in each determination and the highest activity was 26.0 units. The substrate concentration (mM) is discussed in the text.

Fig. 2. Influence of sodium taurocholate on the adsorption of pancreatic lipase. ●, ■ and ▲ are the "adsorbed activity" at 3.3, 6.7 and 33 mM substrate, respectively. Total enzyme protein was 8.5 μg in each determination. For experimental details, see text.

adsorption of the enzyme to the substrate surface. Addition of sodium taurocholate to an assay mixture that had been allowed to react without it increased the activity but never completely restored it. The enzyme was adsorbed to diolein to the same extent as to triolein (olive oil). The hydrolysis of diolein (mixture of 1,2- and 1,3-diolein), however, proceeded more slowly than that of triolein.

It is evident that the adsorption of pancreatic lipase is reversible and is not dependent on the sodium taurocholate concentration, while the lipolytic activity, however, is quite dependent on it. This leads one to believe that the sodium taurocholate is adsorbed to the substrate surface. This may be envisioned in the following manner. The gum arabic in the substrate homogenate forces the fatty acid chains into the center of the micelle, thereby leaving the ester groups at the surface. The sodium taurocholate is then adsorbed providing a molecular template for the enzyme as it approaches the substrate surface. This template in itself may facilitate transformational changes in parts of the protein structure which in turn may provide a more accessible enzymatic site. This concept in general has been described by SCHULMAN AND FRASER[6]. They found that some proteins coat the micellar lipid surfaces and can do so reversibly without loss of activity in some instances. Activation of the pancreatic lipase could be described as a building of an optimum layer of sodium taurocholate on the substrate surface. Inhibition would occur if the sodium taurocholate layer was too thick and in effect pushed the enzyme too far from the surface of the substrate to be active. It is improbable that the sodium taurocholate causes any large phase transitions in the protein molecule because this would most likely result in a drastically changed amount of binding to the olive oil surface, which was not observed.

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Observations of the effect of diethylnitrosamine on glucuronide formation

Glucuronides are formed enzymatically, *in vivo* and *in vitro*, by the transfer of glucuronic acid from UDP-glucuronic acid to a wide range of acceptors in the presence of UDP-glucuronyltransferase (UDP-glucuronate glucuronyltransferase, EC 2.4.1.17), a microsomal enzyme mainly found in liver. In studies of this enzyme *in vitro* the acceptor *o*-aminophenol is often used, since *o*-aminophenol glucuronide formed may be measured in the presence of excess *o*-aminophenol by the sensitive method of LEVVY AND STOREY[1]. GREENWOOD AND STEVENSON[2] demonstrated that the formation of *o*-aminophenol glucuronide by rat liver preparations *in vitro* was increased by the administration of the hepatocarcinogen, diethylnitrosamine, *in vivo* and that the addition *in vitro* of this agent to rat liver homogenate and microsomal suspensions also increased glucuronide formation. Further studies[3] revealed that the stimulatory effect of diethylnitrosamine is limited to the liver of rats and the glucuronide acceptors *o*-aminophenol and paracetamol. The effect is most striking in liver preparations from the GUNN[4] rat which has a persistent unconjugated hyperbilirubinaemia and low hepatic UDP-glucuronyltransferase activity to a wide range of acceptors[5]. The presence of diethylnitrosamine in assays from this strain of rat increases apparent *o*-aminophenol glucuronide formation to the same enhanced level found in similarly treated preparations from normal rats. In this study we report that *o*-aminophenol glucuronide formation is strikingly increased *in vitro* by diethylnitrosamine in assays with crude and partially purified hepatic UDP-glucuronyltransferase preparations[6] from hypophysectomized and thyroidectomized rats as well as Gunn rats. The effect of diethylnitrosamine is further investigated and evidence presented which suggests that its action is on the enzyme preparation rather than on the substrate.

Diethylnitrosamine, UDP-glucuronate as ammonium salt, both stated to be 98–100% pure, were obtained from Sigma Chemical Co. St. Louis, Mo., U.S.A.. *o*-Aminophenol was resublimed from commercially available *o*-aminophenol. *o*-Aminophenol glucuronide was a gift from Professor R. T. Williams. All other chemicals were obtained from commercial sources in the highest purity available and not further purified. Animals used in this study were as previously described[6]. The cat and goat specimens were obtained from healthy animals of unknown pedigree. These latter animals were killed with intravenous sodium pentobarbital. All others were killed by cervical dislocation. Livers were rapidly removed and a 25% homogenate in 0.14 M