reported in table 2 has any effect on these pathological or physiological conditions. In conclusion, monofluoromethyl histidine represents a safe nontoxic means to deplete whole body histamine stores. More will be cerntainly be heard about the pharmacology of this compound.

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Synergistic effect of AMP and fructose 2,6-bisphosphate on the protection of fructose 1,6-bisphosphatase against inactivation by trypsin[1]

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Summary. The rate of inactivation of chicken liver fructose 1,6-bisphosphatase by trypsin is reduced if the digestive reaction is conducted in the presence of AMP or fructose 2,6-bisphosphate. The effects of these 2 compounds are synergistic. Although fructose 1,6-bisphosphate does not protect the enzyme against tryptic inactivation, it can enhance the effect of AMP. Selective modification of the AMP allosteric site of fructose 1,6-bisphosphatase with pyridoxal-P and $NaBH_4$ renders the enzyme more resistant to tryptic inactivation, but the modified enzyme is no longer responsive to the protective effect of AMP.

Fructose 1,6-bisphosphatase (Fru-P_2ase) (EC 3.1.3.11) is a key enzyme in gluconeogenesis. This enzyme is extremely sensitive to allosteric inhibition by AMP as well as competitive inhibition by fructose 2,6-bisphosphate (Fru-2,6-P_2) and the inhibitory effects of these 2 compounds are synergistic[2,3]. It has been previously reported that the rate of inactivation of chicken liver Fru-P_2ase by trypsin was significantly reduced if the digestive reaction was carried out in the presence of AMP[4]. We now report that Fru-2,6-P_2 can also protect this enzyme against tryptic inactivation and that the protective effects of AMP and Fru-2,6-P_2 are synergistic.

Materials and methods. Bovine pancreatic trypsin, yeast glucose-6-P dehydrogenase and phosphoglucose isomerase, and other chemicals were all purchased from Sigma Chemical Company, Saint Louis, MO, USA. Fru-P_2ase was purified from chicken livers by the method previously described[5]. The activity of Fru-P_2ase was measured spectrophotometrically by following the formation of NADPH at 340 nm in a coupled reaction. Unless otherwise stated, the assay mixture (1 ml) contained 50 mM Tris-HCl buffer (pH 7.4), 0.1 mM EDTA, 0.15 mM $NADP^+$, 0.1 M KCl, 2 mM $MgCl_2$, 0.1 mM Fru-1,6-P_2, 1 unit each of phosphoglucose isomerase and glucose-6-P dehydrogenase, and an appropriate amount of Fru-P_2ase. The assay mixture without substrate was preincubated in a cuvette at 25 °C for 2 min. The reaction was initiated by the addition of substrate. The concentration of purified Fru-P_2ase was determined by its extinction coefficient at 280 nm[5]. Fru-2,6-P_2 was chemically synthesized by the method of Pilkis et al.[6]. The concentration of Fru-2,6-P_2 was determined by incubating an aliquot of Fru-2,6-P_2 stock solution at pH 2.5 for 30 min at 28 °C and assaying the amount of fructose-6-P formed using glucose-6-P dehydrogenase and phosphoglucose isomerase[6]. The Fru-2,6-P_2 preparation used in this study contained no detectable fructose-6-P, glucose-6-P, and Fru-1,6-P_2.

Results. Figure 1 shows the time course of changes of Fru-P_2ase activity on modification with trypsin. Under the condition described in the legend of this figure, the time required for inactivation of 50% of Fru-P_2ase activity was approximately 30 min. This increased to about 60 min or 80 min if the digestive reaction was carried out in the presence of 0.12 mM AMP or 0.12 mM Fru-2,6-P_2, respectively. If digestion with trypsin was performed in the presence of both 0.12 mM AMP and 0.12 mM Fru-2,6-P_2, more than 80% of Fru-P_2ase activity still remained even after 200 min. Table 1 shows that the protective effects of both AMP and Fru-2,6-P_2 decreased markedly if digestion with trypsin was carried out at higher pH. It also shows that Fru-1,6-P_2 failed to protect Fru-P_2ase against tryptic inactivation, but it significantly enhanced the protective effect of AMP.

Figure 2 shows that treatment of Fru-P_2ase with pyridoxal-P in the presence of Fru-1,6-P_2 followed by reduction with $NaBH_4$ resulted in irreversible desensitization of the enzyme to allosteric inhibition by AMP with only slight loss

of catalytic activity. These data are in agreement with the previous reports[7,8]. This selective modification of the allosteric site rendered the enzyme more resistant to tryptic inactivation. As shown in table 2, the time required for 50% inactivation of the modified form of Fru-P_2ase was approximately 100 min, compared to about 30 min required for 50% inactivation of the native enzyme. Table 2 also shows that the rate of inactivation of the modified enzyme by trypsin was only very slightly altered in the presence of 0.6 mM AMP, while this same concentration of AMP increased the time required for 50% inactivation of the native enzyme by about 6-fold. The modified enzyme was still protected by Fru-2,6-P_2 against inactivation by trypsin.

Discussion. It has been previously reported that the rate of inactivation of Fru-P_2ase by trypsin can be reduced if the digestive reaction is conducted in the presence of AMP[4]. We now find that Fru-2,6-P_2 can also protect this enzyme against tryptic inactivation. Like the inhibitory effects of AMP and Fru-2,6-P_2 on Fru-P_2ase[2,3], the protective effects of these 2 compounds against tryptic inactivation are also synergistic. The basis for this protection by these 2 compounds is not known. In the case of Fru-2,6-P_2, it is evidently not due to the protection of the catalytic site since the substrate, Fru-1,6-P_2, fails to prevent the inactivation even when added at the concentration as high as 0.5 mM. It is possible that AMP and Fru-2,6-P_2 protect Fru-P_2ase against tryptic digestion by changing the enzyme conformation. The synergistic effect of these 2 compounds suggests that they may mutually enhance the affinity of the enzyme for each other or that they jointly induce an unique conformational state that is highly stable to tryptic digestion. Although Fru-1,6-P_2 does not protect the enzyme against tryptic inactivation, it markedly potentiates the protective effect of AMP. This may be explained by the previous observation that the binding of Fru-1,6-P_2 to Fru-P_2ase enhances the affinity of the enzyme for AMP[9]. The lysyl residues at the allosteric site of Fru-P_2ase can be

Table 1. Effect of pH on the protection of Fru-P_2ase against tryptic inactivation by AMP and Fru-2,6-P_2 *

Additions	% Residual activity after digestion with trypsin for 110 min performed at pH 7.5	pH 8.7
1. None	13.2	10.7
2. 0.12 mM AMP	26.1	13.2
3. 0.12 mM Fru-2,6-P_2	33.4	15.9
4. 0.12 mM each of AMP and Fru-2,6-P_2	87.3	35.9
5. 0.12 mM Fru-1,6-P_2	13.6	10.4
6. 0.12 mM each of AMP and Fru-1,6-P_2	52.4	26.3
7. 0.5 mM Fru-1,6-P_2	13.9	10.1
8. 0.5 mM Fru-2,6-P_2	84.8	33.4
9. 0.5 mM AMP	51.2	24.4

* Digestion with trypsin was performed by incubating Fru-P_2ase (2 mg/ml) at 25 °C in 0.1 M Tris-HCl buffer (pH 7.5 or 8.7) at the ratio of Fru-P_2ase to trypsin of 80:1 (w/w) with or without AMP, Fru-1,6-P_2, and Fru-2,6-P_2 as indicated. After 110 min incubation, aliquots were removed, diluted, and immediately assayed for Fru-P_2ase activities as described in 'Materials and methods'.

Table 2. Inactivation of native and pyridoxal-P-modified Fru-P_2ase by trypsin under various conditions*

Additions	Estimated time for 50% inactivation (min) Native enzyme	Modified enzyme
1. None	31	104
2. 0.12 mM AMP	62	107
3. 0.12 mM Fru-2,6-P_2	79	305
4. 0.6 mM AMP	183	122
5. 0.12 mM each of AMP and Fru-2,6-P_2	362	334

* Digestion with trypsin was performed at pH 7.5 as described in the legend of figure 1. At the time intervals, aliquots were removed, diluted and immediately assayed for Fru-P_2ase activities to determine the time required for 50% inactivation.

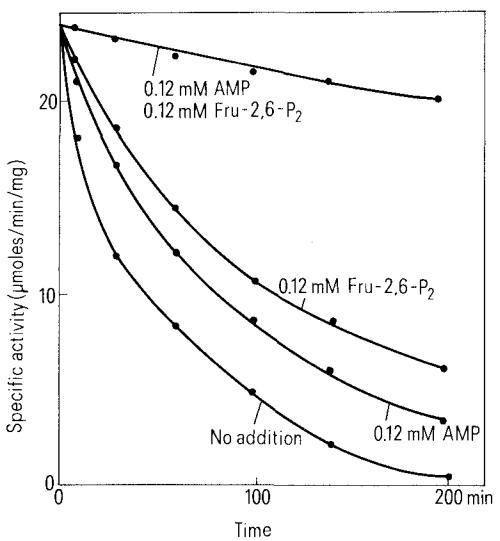

Figure 1. Effect of AMP and Fru-2,6-P_2 (on the inactivation of Fru-P_2ase) by trypsin. Digestion with trypsin was performed by incubating Fru-P_2ase (2 mg/ml) at 25 °C in 0.1 M Tris-HCl buffer (pH 7.5) at the ratio of Fru-P_2ase to trypsin of 80:1 (w/w) with or without AMP and Fru-2,6-P_2 as indicated. At the time intervals indicated, aliquots were removed, diluted, and immediately assayed for Fru-P_2ase activities as described in 'Materials and methods'. Throughout this study, the maximum amounts of AMP and Fru-2,6-P_2 that were introduced into the assay mixture were negligible for Fru-P_2ase activity.

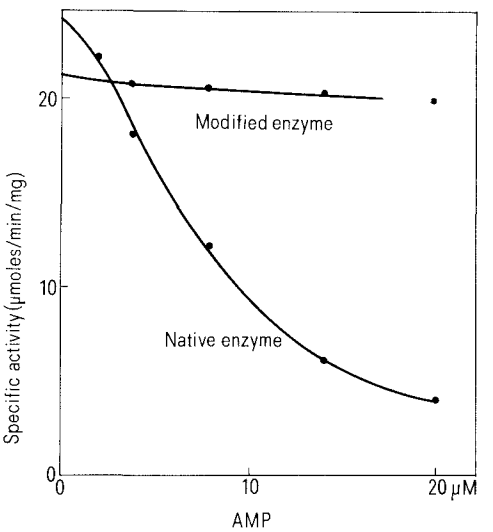

Figure 2. Inhibition of native and pyridoxal-P-treated Fru-P_2ases by varying concentrations of AMP. Treatment of Fru-P_2ase with pyridoxal-P was performed as previously described[7]. The enzyme (2 mg/ml) was incubated with 1 mM pyridoxal-P in the presence of 5 mM Fru-1,6-P_2 at 25 °C for 20 min in 50 mM Na borate buffer (pH 8.0) containing 0.5 mM EDTA. The solution was cooled to 0 °C and 2 drops of octyl alcohol was added to avoid foaming. A freshly prepared solution of $NaBH_4$ was then added slowly to the point of decoloration of the reaction mixture. The solution was then dialyzed at 4 °C for 15 h against 1000 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 0.1 mM EDTA (2 buffer changes). Fru-P_2ase activity was assayed as described in 'Material and methods' with varying concentrations of AMP as described.

selectively modified with pyridoxal-P and $NaBH_4$ without significant loss of catalytic activity. This modified enzyme becomes more resistant to tryptic inactivation. It is possible that the binding of pyridoxal-P to the enzyme may induce a conformational change which has little effect on catalytic activity but has significant effect on tryptic digestion. It is also possible that the lysyl residues at the allosteric site may be the site of tryptic digestion since trypsin is known to cleave peptide linkage in protein whose carbonyl groups are contributed by lysine[10]. It has been suggested that Fru-2,6-P_2 may also interact with AMP allosteric site[11], but the fact that Fru-2,6-P_2 can still protect the modified enzyme against tryptic inactivation indicates that the protective effect of Fru-2,6-P_2 is not due to the binding to the AMP allosteric site.

The protective effect of AMP or Fru-2,6-P_2 decreased markedly if the digestive reaction was conducted at higher pH. These observations appear to be in agreement with the fact that inhibition of Fru-P_2ase activity by AMP or Fru-2,6-P_2 decreased greatly at higher pH[3,5]. It is suggested that at higher pH Fru-P_2ase may decrease affinity for AMP and Fru-2,6-P_2 or the enzyme may become less vulnerable to conformational changes by these compounds.

In this study, we have found that the rate of inactivation of yeast glucose-6-P dehydrogenase or phosphoglucose isomerase by trypsin is not altered by the addition of 0.5 mM AMP, or 0.5 mM Fru-2,6-P_2, or both (data not shown). This indicates that the protection of Fru-P_2ase against tryptic inactivation by AMP and Fru-2,6-P_2 is not due to the inhibition of trypsin by these compounds.

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The effect of jaundiced sera and bile salts on cultured beating rat heart cells

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Summary. Jaundiced serum from common bile duct ligated rats, added to cultured heart cells, decreased the beating rate, caused an early cessation of beating and production of higher levels of lactate in the media. Deoxycholate and cholate are the main bile acids in jaundiced serum; deoxycholate caused similar effects, which suggests that it is the toxic substance responsible for heart function alterations seen in patients with severe jaundice.

In liver disease associated with jaundice, the blood levels of bilirubin and bile acids are elevated. Bile acids are toxic in a variety of ways. They were shown to inhibit Na-K and Mg ATPases activities, oxygen uptake and protein synthesis in the small intestine of the rat[1], they disrupt lysozymes[2] and produce hemolysis[3,4]. Following the ligation of the bile ducts there was a significant reduction in the response of skeletal muscle to noradrenalin[5], and impaired cardiovascular responsiveness to phenoxybenzamine and saralazin[6]. Bile acids also cause bradycardia, negative ionotropism, arrythmia and cardiac arrest of the isolated heart[7-10].

Biochemical, physiological and electrophysiological studies have shown the beating heart cells in tissue culture to be a useful model for the studies of cardiac function and metabolism[11]. The present work deals with the effects of bile acids and jaundiced serum from common bile duct ligated (CBDL) rats on beating heart cells in tissue culture.

Materials and methods. For the preparation of the sera, Wistar rats weighing 200–220 g were used. The jaundiced serum was obtained 6 days after the rats were operated and CBDL, while the controls were sham operated. Rat heart cells were prepared as previously described[11,12], with the media containing 5% calf fetal serum and 5% horse serum. Whenever the effect of jaundiced serum was studied, the horse serum was omitted and the test control and experimental rat sera were added. Contraction rates were determined visually under the microscope, at various time intervals after the addition of the jaundiced serum or bile

Table 1. Effect of jaundiced sera from bile duct ligated rats on contraction rate of heart cells in tissue culture

Time after addition of sera (h)	Contractions per min: Control serum 5%	Control serum 10%	BDL serum 5%	p	BDL serum 10%	p
−1	146 ± 10	151 ± 6	148 ± 10		150 ± 11	
2	139 ± 11	132 ± 8	128 ± 11		124 ± 15	
4	140 ± 14	138 ± 10	118 ± 13		94 ± 14	< 0.01
8	138 ± 13	140 ± 12	94 ± 11	< 0.01	70 ± 16*	< 0.01
12	128 ± 17	133 ± 11	68 ± 12*	< 0.01	59 ± 10*	< 0.01
22	106 ± 18	114 ± 15	32 ± 6*	< 0.01	13 ± 6*	< 0.01
30	95 ± 16	96 ± 11	21 ± 5*	< 0.01	NB	< 0.01
48	58 ± 9	64 ± 8	NB	< 0.01	NB	< 0.01

Experimental details in text. Data are mean ± SEM (N = 15). NB, not beating; *irregular beating; p, significance between control and BDL groups.