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.

1 This work was supported by grant RR-8006 from the General Research Branch, Division of Research Resources, NIH (USA).
2 Van Schaftingen, E., and Hers, H.G., Proc. natl Acad. Sci. USA *78* (1981) 261.
3 Pilkis, S.J., El-Maghrabi, M.R., McGrane, M.M., Pilkis, J., and Claus, T.H., J. biol. Chem. *256* (1981) 1148.
4 Han, P.F., Murthy, V.V., and Johnson, J., Archs Biochem. Biophys. *173* (1976) 293.
5 Han, P.F., and Johnson, J., in: Methods in Enzymology, p. 334. Ed. W.A. Wood. Academic Press, New York 1982.
6 Pilkis, S.J., El-Maghrabi, M.R., Pilkis, J., Claus, T.H., and Cumming, D.A., J. biol. Chem *256* (1981) 2619.
7 Colombo, G., Hubert, E., and Marcus, F., Biochemistry *11* (1972) 1798.
8 Cruz, Z.M., Tanizaki, M.M., El-Dorry, H.A., and Bacila, M., Archs Biochem. Biophys. *198* (1979) 424.
9 Sarngadharan, M.G., Watanabe, A., and Pogell, B.M., Biochemistry *8* (1969) 1411.
10 Lehninger, A.L., Principles of Biochemistry. Worth Publishers, Inc., New York 1982.
11 Pilkis, S.J., El-Maghrabi, M.R., McGrane, M.M., Pilkis, J., and Claus, T.H., J. biol. Chem. *256* (1981) 11489.

0014-4754/83/111305-03$1.50 + 0.20/0

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

E. Bogin, O. Better and I. Harari

Kimron Veterinary Institute, Bet-Dagan (Israel), Rambam Medical Center, Haifa (Israel), and University of California, Los Angeles (California 90024, USA), February 28, 1983

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.

Table 2. Effect of bile salts on the contraction rate of rat heart cells in tissue culture

Time after addition (h)	Contractions per min Control	Taurodeoxycholic acid (μmoles/l) 20	40	60	Glycocholic acid (μmoles/l) 20	40	60
−1	85 ± 5	86 ± 6	84 ± 7	87 ± 6	85 ± 6	84 ± 7	87 ± 5
2	85 ± 6	67 ± 7*	NB	NB	125 ± 11**	122 ± 13**	145 ± 10
3	84 ± 6	65 ± 7**	NB	NB	121 ± 13**	118 ± 11**	141 ± 12**
4	84 ± 5	57 ± 6**	NB	NB	81 ± 7	78 ± 6	85 ± 7
6	80 ± 8	55 ± 8**	NB	NB	71 ± 8	77 ± 8	73 ± 8
8	76 ± 7	50 ± 6**	NB	NB	68 ± 6	62 ± 6	64 ± 5
12	74 ± 6	34 ± 5**	NB	NB	67 ± 5	62 ± 7	53 ± 6*
24	52 ± 7	21 ± 6**	NB	NB	48 ± 8	43 ± 9	37 ± 7*

Experimental details in text. *$p < 0.05$; **$p < 0.01$ ($N = 10$).

acids. For the evaluation of liver damage, the level of glutamic-oxalacetic transaminase (GOT; EC.2.6.1.1.) was determined spectrophotometrically[13]. Bile salts were determined according to the method of Iwata and Yamasaki[14]. Lactic acid formed in the media was determined spectrophotometrically[13].

Results and discussion. Beating heart cells in tissue culture, which have been shown to be a good model for cardiac cell function and metabolism[11,15] were strongly affected by jaundiced sera and bile acids. Jaundiced serum obtained from rats following CBDL, contained 300–360 μmoles/l of both cholic (CA) and deoxycholic (DCA) acids, in comparison to 10 μmoles/l in serum from sham operated controls. There was distinct liver damage, as could be seen from the GOT level which was 96 ± 12 U/l vs 28 ± 4 in the controls. Addition of 5 or 10% CBDL serum to the media had no significant effect for the 1st 2 h. After 4 h, however, a marked inhibition in the beating rate was seen, followed by earlier cessation (table 1). At 22 h after the addition of the sera, there was about 70% and 89% inhibition with cells in the medium containing 5% and 10% CBDL serum respectively. The beating of these cells started to be irregular at 8 h with 10% CBDL serum and at 12 h with 5% CBDL. The effect of bile acids on the beating rate is shown in table 2. Glycocholic acid at concentrations of 20, 40 and 60 μmoles/l caused a significant stimulation of the beating rate, which lasted up to 3 h after which time it returned to the rate of the controls. Tauro-DCA was more toxic than glyco-CA. Levels of 40 and 60 μmoles/l were very toxic, quickly causing a cessation of beating. At concentrations of 20 μmoles/l, there was a significant inhibition starting at the 1st reading of 2 h; this increased with time, reaching 60% at 24 h in comparison to the controls. The effect of the bile acids on heart cell metabolism, as evaluated by the pH change and lactic acid formation in the media is shown in table 3. Sera from CBDL rats and tauro-DCA but not glyco-CA led to a lower pH of the media and increased the level of lactate. The largest changes in pH and lactate formation were seen in the media containing 40 and 60 μmoles/l DOC.

The present study demonstrated that bile salts at concentrations present in the blood of jaundiced rats affected beating heart cells in tissue culture, causing a slowing down of the beating rate, irregular contractions and an earlier cessation of beating. Jaundiced serum obtained from rats following CBDL contains high levels of both CA and DOC[10], but it seems that DOC rather than CA is the toxic substance responsible for the effects seen.

The mode of action by which this substance exerts its toxic effect could be by its effects on biological membranes; it has been shown to cause disruption of cell membranes, swelling of mitochondria and sarcoplasmic reticulum[3] and to affect oxidative phosphorylation[16] by inhibiting oxygen uptake and inorganic phosphorus esterification. The decreased mitochondrial oxidative phosphorylation without glycolysis being affected could lead to the accumulation of lactate in the medium and the lowering of the pH. Lower activity of oxidative phosphorylation leads to lower cellular ATP levels, which if decreased by 10–20% will cause cessation of beating[17].

Table 3. Effect of bile salts and BDL serum on rat heart cell metabolism as evaluated by changes in pH of the media and lactic acid content (at 24 h after addition of the serum or bile salts)

Group or treatment	Beating	Change from control (X ± SEM) pH (units)	Lactic acid (μmoles/ml)
Control (horse serum)	+	0.0	0.0
Control (rat serum)	+	− 0.04 ± 0.01	0.06 ± 0.01
BDL (rat serum)	+	− 0.03 ± 0.02*	0.44 ± 0.03*
Na-DOC 10 μM	+	− 0.23 ± 0.03*	0.71 ± 0.1*
Na-DOC 20 μM	+	− 0.61 ± 0.04*	1.45 ± 0.12*
Na-DOC 40 μM	−	− 0.61 ± 0.04*	1.61 ± 0.16*
Na-DOC 60 μM	−	− 0.51 ± 0.03*	0.90 ± 0.05*
Glycocholate 20 μM	+	+ 0.05 ± 0.01	0.05 ± 0.01
Glycocholate 40 μM	+	+ 0.04 ± 0.01	0.06 ± 0.01
Glycocholate 60 μM	+	+ 0.08 ± 0.02	0.06 ± 0.01

Experimental details in text. Experimental additions were made to cultures containing horse serum. *$p < 0.01$ ($N = 6$).

1 Parkinson, T.M., and Olson, J.A., Life Sci. *3* (1964) 107.
2 Weissman, G., Biochem. Pharmac. *14* (1965) 525.
3 Berliner, F., and Schoenheimer, R., J. biol. Chem. *129* (1938) 525.
4 Weissman, G., and Keiser, H., Biochem. Pharmac. *14* (1965) 537.
5 Bomzon, L., Wilton, P.B., and McCalden, T.A., Clin. Sci. molec. Med. *55* (1978) 109.
6 Lunzer, M.R., Manghani, K.K., Newman, S.P., Sherlock, S.V.P., Bernard, A.G., and Ginsburg, J., Lancet *2*, (1975) 382.
7 Jaubert, P., Clin. exp. Pharmac. Physiol. *5* (1977) 1.
8 Jaubert, P., Clin. exp. Pharmac. Physiol. *5* (1978) 9.
9 Wakim, K.G., Essex, H.E., and Mann, F.C., Am. Heart J. *18* (1939) 171.
10 Wakim, K.G., Essex, H.E., and Mann, F.C., Am. Heart J. *20* (1940) 486.
11 Harary, I., and Farley, B., Exp. Cell Res. *29* (1963) 451.
12 Harary, I., Hoover, F., and Farley, B., Meth. Enzym. *32* (1974) 740.
13 Bergmeyer, H.U., and Gawehn, K., Methods in Enzymatic Analysis, 2nd edn. Academic Press, New York and London 1974.
14 Iwata, T., and Yamasaki, K., J. biol. Chem., Tokyo *56* (1964) 424.
15 Harary, I., in: The Cardiovascular System, p.43. Ed. R.M. Berne. 1979.
16 Ernster, L., in: Kernicterus, p.171. Ed. A. Sass-Korfisak. University of Toronto Press, Toronto 1961.
17 Baltaceano, G., and Vasilu, C., C. r. Soc. Biol. *5* (1934) 1550.

0014-4754/83/111307-02$1.50 + 0.20/0