homogenates. No clear trends emerged in the levels of Tau, Bet or Gly in the rest of the tissue, although Ala showed a dependence on salinity after 2 weeks' incubation. This suggests that these metabolites may not be the major osmoregulatory solutes in every organ in *T. watlingi*, although their presence in fairly high concentrations requires that they contribute significantly to the overall osmotic equilibrium. It is also possible that some organs do not achieve osmotic equilibrium with the haemolymph during this period, or that they do not maintain constant volume under conditions of osmotic stress.

In foot muscle the levels of the major organic solutes begin to respond to alterations in environmental salinity within two days. As *T. watlingi* is an estuarine mollusc, and therefore subject to significant salinity fluctuations under natural conditions, this response is of physiological importance. Notwithstanding this, specimens of *T. watlingi* are often found buried in the sand or mud with only part of the shell protruding, and their initial response to environmental stress may be valve closure. It is known that isolated tissue from these bivalves can withstand long periods of anoxia without addition of nutrients[6,7].

Tau, Bet, Gly and Ala have all been found in a wide range of marine organisms[8]. In a number of organisms, including many species of molluscs[9–13], one or more of these compounds has been found to play a role in osmoregulation. Examination of a number of other molluscs by ^{13}C NMR reveals that Tau, Bet and some polyols are present in all species, with Gly being present in some[3]. Thus, it is likely that the major organic solutes observed in ^{13}C (and ^{1}H) NMR-studies of tissue from marine organisms will be those involved, at least to some extent, in osmoregulation. These techniques should, therefore, be particularly useful for the study of osmoregulation in marine organisms.

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Purification and partial characterization of two lectins from *Momordica charantia*[1]

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Summary. 2 different lectins have been purified from the seeds of *Momordica charantia* by gel-filtration and ion-exchange chromatography. These 2 lectins appear to be composed of 2 subunits of 26,000 daltons. Protein fraction I, but not II, showed agglutinating activity toward human type-O red blood cells. The amino acid compositions and amino-terminal sequences of these two homologous proteins are quite different.

The fruit of *Momordica charantia* is widely used in the orient, although the seeds are not eaten. The D-galactose-binding agglutinin from *Momordica charantia* has been shown to agglutinate human type-O red blood cells, but not Yoshida sarcoma cells[2]. Recently, toxic momordin and non-toxic momordica agglutinin have also been separated by CM-cellulose chromatography, and the momordin inhibits protein biosynthesis of Ehrlich ascites tumor cells[3]. In this report, 2 lectins have been purified from the seeds of *Momordica charantia*, and their molecular weights, amino acid compositions and aminoterminal sequences of 27 residues have been determined.

Materials and methods. The seeds of *Momordica charantia* were obtained from Chan Man Hop Seed Co., Hong Kong. The proteins were isolated as previously described[4]. Hemagglutination assays were performed in microtiter plates with human type-O red blood cells[5]. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate was performed on 12.5% slab gel in Tris-glycine buffer, pH 8.3[6]. The gels were stained for protein with Coomassie brilliant blue and for carbohydrate with periodic acid-Schiff reagent[7].

The proteins were hydrolyzed in 6 N HCl at 110 °C for 24, 48 and 72 h, and the hydrolysates were analyzed with an automatic amino acid analyzer (Beckman 121). Cysteine and/or half-cysteine were determined as cysteic acid after performic acid oxidation[8]. Automatic Edman degradations were performed with the Beckman protein sequencer using N, N′-dimethylallylamine buffer and single acid cleavage[9,10]. Phenylthiohydantoin-amino acids were identified by GLC[11], TLC[12], and/or amino acid analysis after back hydrolysis with 6 N HCl or 56% HI[13]. Phenylthiohydantoin-arginine was also identified by the phenanthrene quinone spot test[14].

Results. The crude protein extract was chromatographed on a column of DEAE-Sephadex (figure 1,a) followed by gel filtration on a Sephadex G-150 column (figure 1,b). The proteins under peak GI were further separated into fractions I and II on CM-cellulose column using a linear gradient of sodium phosphate buffer (figure 1,c). The

proteins under peak G2 were shown to be low-molecular-weight storage proteins, and have been described previously[4].

The proteins from DEAE-A50 and fraction G1 on G-150 column had hemagglutination titers of 1280 and 320, respectively, toward human type-O red blood cells. The purified protein fraction I agglutinated human type-O red blood cells at the concentration of about 2–4 μg/ml, while protein fraction II did not show agglutination even at 40 μg/ml. This hemagglutination could be inhibited by 10 or 25 mM D-galactose.

The reduced samples of proteins were run on SDS-polyacrylamide gel electrophoresis, and the gels were stained for protein and carbohydrate. Both protein fractions I and II showed single protein band corresponding mol. wt of 26,000 daltons (figure 2). Both proteins were also periodic acid-Schiff positive, indicating the presence of carbohydrate (data are not presented). The mol. wt of proteins under peak G1 (figure 1,b) was estimated to be approximately 49,000 daltons by gel-filtration on a calibrated Sephadex G-150 column. These results indicate that both proteins consist of 2 subunits of approximately 26,000 daltons.

The amino acid composition of both proteins (table 1) was calculated from the molar ratio and the assumed mol. wt of 26,000 daltons. These 2 proteins contain quite different amino acid compositions, although they have very similar mol. wts. The amino-terminal sequences of 27 residues of these 2 proteins were deduced from 2 runs of automatic Edman degradations. The residues identified are summarized in table 2 and both sequences are compared in figure 3. 13 amino acid differences are observed among first 27 residues compared.

Both protein fractions I and II showed very similar near-UV absorption spectra with a typical maximum around 280 nm and the ratio of A_{280} to A_{260} being 2.0. These spectra are quite different from those of momordica storage proteins reported previously[4].

Discussion. 2 D-galactose-binding lectins were purified from *Momordica charantia,* and only protein fraction I, not II, agglutinated human red blood cells. On the basis of hemagglutinating activities and the behavior on CM-cellulose chromatography, the protein fractions I and II appear

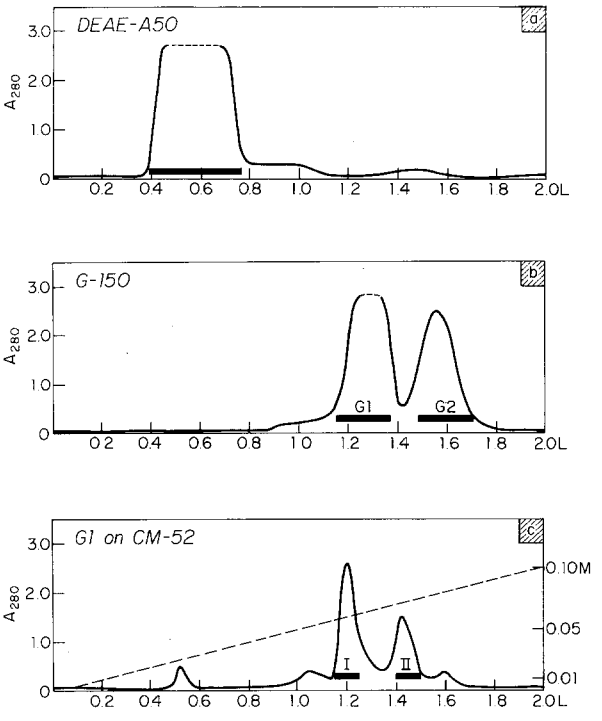

Fig. 1. Purification of 2 momordica lectins. The proteins were extracted from the decorticated, defatted meal with 0.9% NaCl and precipitated by 95% saturation of ammonium sulfate. The precipitate suspension was left overnight at 4 °C, and then centrifuged at 25,500× g for 1 h. The protein precipitate was dissolved in water, and dialyzed against running water for 20 h. Some solid precipitate was removed by centrifugation, and the crude protein extract was then purified by gel filtration and ion-exchange chromatography at 4 °C. *a* DEAE-Sephadex chromatography: The column (5× 70 cm) was preequilibrated and eluted with 5mM sodium acetate, pH 5.8. *b* Sephadex G-150 column: The pooled protein fractions was chromatographed on a column (2.6× 90 cm) of Sephadex G-150 in 0.05 M Tris-HCl, 0.1 M NaCl, pH 7.6 buffer. *c* CM-cellulose chromatography: The column (5× 60 cm) was pre-equilibrated and first eluted with 100 ml of the initial gradient. The column was then developed with a linear gradient of sodium phosphate buffer (pH 6.5, 0.01 M–0.10 M, 950 ml each).

Table 1. Amino acid composition of 2 momordica lectins

	Protein fraction I		Protein fraction II	
Amino acids	Calculated values	Integer	Calculated values	Integer
Lysine	12.11[a]	12	7.86	8
Histidine	2.07	2	2.21	2
Arginine	18.38	18	10.21	10
Aspartic Acid	23.83	24	27.46	27
Threonine	16.13[b]	16	20.69	21
Serine	16.61[b]	17	14.54	15
Glutamic Acid	19.08	19	19.41	19
Proline	12.11	12	17.54	18
Glycine	14.24	14	9.25	9
Alanine	17.68	18	22.70	23
1/2 Cystine	trace[c]	–	trace	–
Valine	12.86[d]	13	15.27	15
Methionine	1.83	2	trace	–
Isoleucine	13.99[d]	14	11.23	11
Leucine	23.46	23	15.09	15
Tyrosine	9.76	10	14.23	14
Phenylalanine	6.28	6	13.09	13
Tryptophane[e]				
Total	220.42	220	220.78	220

[a] Average of values for 24-, 48- and 72-h hydrolysis except where indicated. [b] Extrapolated to zero time. [c] Determined as cysteic acid. [d] From 72-h hydrolysis. [e] Not determined.

Table 2. Sequential Edman degradations

Step No.	Protein fraction I Deduced residues	GLC[b]	TLC[c]	AAA[d]	Protein fraction II Deduced residues	GLC	TLC	AAA
1	Asp[a]	D	D	D	Asp	D	D	
2	Val	V	V	V	Val	V	V	
3	Ser	S	S	S*[h]	Asn	N	N	
4	Phe	F_e	F	F	Phe	F	F	
5	Arg	-	R[f]	R	Asp	D	D	
6	Leu	L	L/I[g]	L	Leu	L	L/I	
7	Ser	S	S	S*	Ser	S	S	S*
8	Gly	G	G	G	Thr	T	T	T*
9	Ala	A	A	A	Ala	A	A	A
10	Asp	D	D	D	Thr	T	T	T*
11	Pro	P	P	P	Ala	A	A	A
12	Arg	-	R	R	Lys	K	K	
13	Ser	S	S	S*	Thr	T	T	T*
14	Tyr	Y	Y	Y	Tyr	Y	Y	Y
15	Gly	G	G	G	Thr	T	T	T*
16	Met	M	M	M	Lys	K	K	
17	Phe	F	F	F	Phe	F	F	
18	Ile	I	L/I	I	Ile	I	L/I	
19	Lys	K	K	K	Glu	E	E	
20	Asp	D	D	D	Asp	D	D	
21	Leu	L	L/I	L	Phe	F	F	
22	Arg	-	R	R	Arg	-	R	
23	Asn	N	N	D	Ala	A	A	
24	Ala	A	A	A	Thr	T	T	T*
25	Leu	L	L/I	L	Leu	L	L/I	
26	Pro	P	P	P	Pro	P	P	
27	Phe	F	F	F	Phe	F	F	

[a] The single and 3-letter abbreviations for the amino acids are those suggested by the IUPAC-IUB Commission on Biochemical Nomenclature[16]. [b] Only those phenylthiohydantoin (PTH)-amino acids extracted with ethyl acetate following conversion were analyzed as silylated and unsilylated derivatives on SP400 column in GLC. [c] PTH-amino acids were run on polyamide sheets (5×5 cm) with a fluorescent indicator in the first solvent of TLC. [d] PTH-amino acids were hydrolyzed with 6 N HCl or 56% HI, and then analyzed on amino acid analyzer (AAA). [e] The sample was analyzed but no PTH-amino acid was detected. [f] PTH-arginine was also identified by the phenanthrenequinone spot test. [g] PTH-leucine and PTH-isoleucine could not be differentiated unambiguously. [h] PTH-serine and PTH-threonine were identified as alanine and α-aminobutyric acid after HI hydrolysis, respectively.

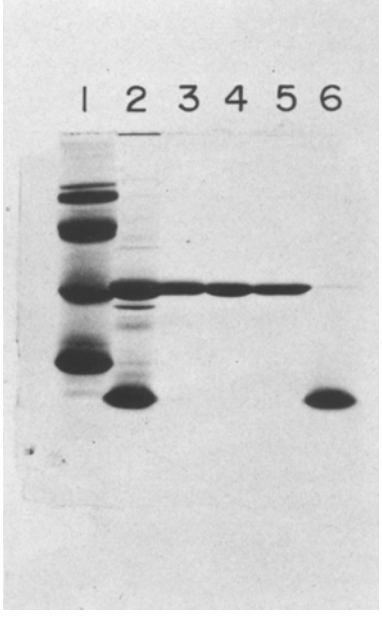

Fig. 2. SDS-polyacrylamide gel electrophoresis of momordica proteins: *1* Mol. wt standards; serum albumin (68,000), ovalbumin (43,000), chymotrypsinogen (25,700) and ribonulease (13,700); *2* protein fraction from DEAE-A50 column (figure 1,a); *3* fraction G1 from G-150 column (figure 1,b); *4* fraction I from CM-52 column (figure 1,c); *5* fraction II from CM-52 column (figure 1,c); and *6* fraction G2 from G-150 column (figure 1,b).

to correspond to the previously reported momordica agglutinin and toxic momordin, respectively[3]. However, the mol. wts and amino acid compositions of both momordica proteins, I and II, determined in this study differ from the published data[3]. The larger mol. wt and higher glutamic acid content of the previously isolated momordica agglutinin[3] might be due to the contaminated momordica storage protein. Momordica storage protein was eluted from CM-cellulose column at similar position as the case of momordica agglutinin and it has an apparent mol. wt of 5500 daltons and very high content (32–34 moles%) of glutamic acid[4]. It may be noted that both protein fractions I and II, like the D-galactose binding sophora agglutinin[2] and ricin E[15], did not bind to sepharose 4B column.

The partial amino acid sequences of both proteins along with their amino acid compositions indicated that these 2 homologous proteins have very different primary structures. 5 threonine residues are present at position nos 8, 10,

	5					10					15	
I.	Asp	Val	Ser	Phe	Arg	Leu	Ser	Gly	Ala	Asp	Pro	Arg	Ser	Tyr	Gly
II.	-	-	Asn	-	Asp	-	-	Thr	-	Thr	Ala	Lys	Thr	-	Thr

	20					25					
I.	Met	Phe	Ile	Lys	Asp	Leu	Arg	Asn	Ala	Leu	Pro	Phe
II.	Lys	-	-	Glu	-	Phe	-	Ala	Thr	-	-	-

Fig. 3. Partial amino acid sequences of 2 momordica lectins: The identical residues are indicated by a hyphen (-). The protein fractions I and II correspond to the previously reported momordica agglutinin and toxic momordin[3].

13, 15, and 24 of protein fraction II while threonine is absent in the first 27 residues of protein fraction I. It will be of interest to define the exact correlation of the structural differences with their distinct agglutinating and toxic properties.

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Ethanol and liver protein synthesis in vivo[1]

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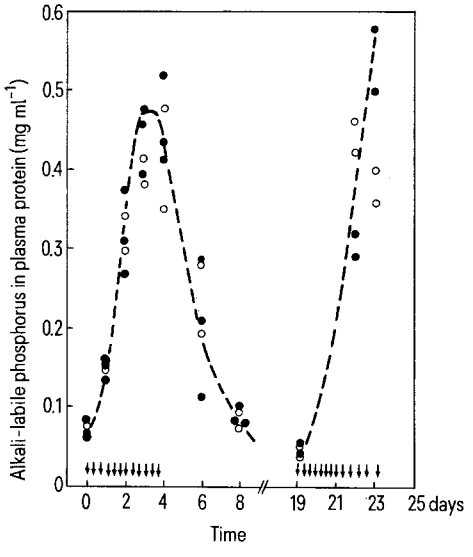
Summary. Ethanol was administered i.p. to adult roosters during hormonally induced vitellogenin synthesis. At moderate doses, ethanol had no influence on the synthesis of vitellogenin nor did it cause alterations in the size distribution of liver polyribosomes.

Although inhibition of liver protein synthesis after acute ethanol administration has been demonstrated in experiments with perfused livers[2,3], liver slices[4] or isolated hepatocytes[5], in vivo effects of ethanol on liver protein synthesis have rarely been studied. While a slight inhibition of albumin synthesis has been observed in rats after an oral administration of ethanol[6,7], a stimulation occurred in animals treated i.p.[6]. The purpose of the present study was to investigate the in vivo effects of acute ethanol administration on specific protein synthesis in avian liver using as a model system the rapid induction of synthesis of a yolk phosphoprotein precursor, vitellogenin, by estrogens[8,9].

Materials and methods. White Leghorn roosters weighing about 1.2 kg were used. The induction of vitellogenin synthesis by estradiol 17β-benzoate and the determination of protein-bound alkali-labile phosphorus in plasma have been described previously[8,9]. Ethanol was administered i.p. as 20% (v/v) solution in saline at designated times. Control animals received saline injections. Blood alcohol was determined enzymatically[10] and liver polyribosomes were isolated and analyzed by sucrose gradient centrifugation as described previously[9].

Results. As shown in the figure, there were no marked differences in plasma levels of vitellogenin in control roosters, and in animals which received repeated injections of ethanol for about 4 days during the initial active period of vitellogenin synthesis (primary and secondary stimulation[8,9]). The maximum concentration of ethanol in the blood achieved after an i.p. injection was about 40 mM and the disappearance of ethanol from the blood took about 6 h. The size distribution of liver polyribosomes was analyzed at 24 h after the estrogen injection and ethanol was administered during this period at 0, 5, 11 and 21 h at a dose of 1.5 g kg^{-1}. In control animals, the polyribosome profiles were similar to those reported previously[10] indicating that a majority of the ribosomes were engaged in active protein synthesis. Treatment of the animals with ethanol was found to have no effect on liver polyribosome profiles in these experiments (data not shown).

Discussion. The frequent finding that the administration of a specific amino acid or a mixture of amino acids partially or entirely prevents the inhibition of liver protein synthesis and the concomitant disaggregation of polyribosomes has suggested that the alterations caused by ethanol are possibly mediated through a limited availability of certain



Plasma levels of phosphoprotein (representing vitellogenin[9]) in roosters during the primary and secondary stimulation. Alkali-labile phosphorus in lipid-free plasma protein was measured following injection of 15 mg kg^{-1} of estradiol 17β-benzoate i.m. at day 0. A 2nd similar injection was given at day 19. (○) roosters receiving saline, (●) roosters receiving ethanol (1.5 g kg^{-1} b.wt.) i.p. at 0, 6, 20, 24, 30, 44, 48, 54, 68, 72, 78 and 92 h after the 1st estrogen injection and at 0, 5, 11, 20, 25, 30, 35, 40, 45, 51, 57, 69, 75 and 81 h after the 2nd estrogen injection (arrows). Each symbol represents a value derived from 1 rooster and determined in triplicate.