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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2 M. Tomita, T. Kurokawa, K. Onozaki, N. Ichiki, T. Osawa and T. Ukita. Experientia *28*, 84 (1972).
3 J.-Y. Lin, M.-J. Hou and Y.-C. Chen, Toxicon *16*, 653 (1978).
4 S.S.-L. Li, Experientia *33*, 895 (1977).
5 R. Kaplan, S.S.-L. Li and J.M. Kehoe, Biochemistry *16*, 4297 (1977).
6 K. Weber and M. Osborn, J. biol. Chem. *244*, 4406 (1969).
7 R.M. Zacharius, T.E. Zell, J.H. Morrison and J.J. Woodlock, Analyt. Biochem. *30*, 148 (1969).
8 C.H.W. Hirs, J. biol. Chem. *219*, 611 (1956).
9 P. Edman and G. Begg, Eur. J. Biochem. *1*, 80 (1967).
10 S.S.-L. Li, J. Hanlon and C. Yanofsky, Biochemistry *13*, 1736 (1974).
11 J. Pisano, T.J. Bronzert and H.B. Brewer, Jr, Analyt. Biochem. *45*, 43 (1972).
12 M.R. Summers, G.W. Smythers and S. Oroszlan, Analyt. Biochem. *53*, 624 (1973).
13 O. Smithies, D. Gibson, E.M. Fanning, R.M. Goodfliesh, J.G. Gilman and D.L. Ballantype, Biochemistry *10*, 4912 (1971).
14 S. Yamada and H.A. Itano, Biochim. biophys. Acta *130*, 538 (1966).
15 T. Mise, G. Funatzu, M. Ishiguro and M. Funatzu, Agric. Biol. Chem. *41*, 2041 (1977).
16 IUPAC-IUB Commission on Biochemical Nomenclature, J. biol. Chem. *241*, 2491 (1966).

Ethanol and liver protein synthesis in vivo[1]

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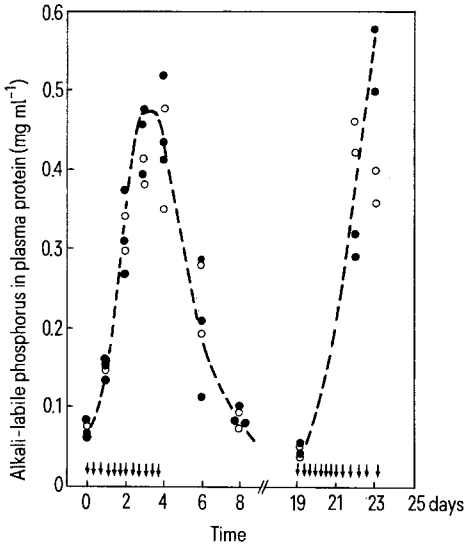
Summary. Ethanol was administered i.p. to adult roosters during hormonally induced vitellogenin synthesis. At moderate doses, ethanol had no influence on the synthesis of vitellogenin nor did it cause alterations in the size distribution of liver polyribosomes.

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.

amino acids in liver cells[2,3,7]. The situation seems to be different in vivo and ethanol may alter the supply of amino acids e.g. through an activation of the pituitary-adrenal system[6]. The rapid activation of protein synthesis in avian liver after estrogen administration is probably a sensitive system to detect the possible effects of ethanol on protein synthesis. In the absence of any effects of ethanol treatment on plasma levels of vitellogenin and on the size distribution of liver polyribosomes under these conditions we conclude that despite of its large effects on oxidative metabolism and substrate usage acute ethanol administration does not influence avian liver protein synthesis in vivo.

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2 M.A. Rothschield, M. Oratz, J. Mongelli and S.S. Schreiber, J. clin. Invest. *50,* 1812 (1971).
3 M. Oratz, M.A. Rothschield and S.S. Schreiber, Ann. N.Y. Acad. Sci. *252,* 51 (1975).
4 A. Perin, G. Scalabrino, A. Sessa and A. Arnaboldi, Biochim. biophys. Acta *366,* 101 (1974).
5 J. Mørland and A. Bessesen, Biochim. biophys. Acta *474,* 312 (1977).
6 G.-S.D. Nadkarni, Biochem. Pharmac. *23,* 389 (1974).
7 K.N. Jeejeebhoy, M.J. Phillips, A. Bruce-Robertson, J. Ho and U. Sodtke, Biochem. J. *126,* 1111 (1972).
8 P.H. Mäenpää and M.R. Bernfield, Biochemistry *8,* 4926 (1969).
9 P.H. Mäenpää, Biochem biophys. Res. Commun. *72,* 437 (1976).
10 E. Bernt and I. Gutmann, in: Methods of Enzymatic Analysis, p. 1499. Ed. H.U. Bergmeyer. Verlag Chemie, Weinheim 1974.

Energetics of myo-inositol transport in *Pseudomonas putida*[1]

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Summary. The effects of specific inhibitors on the high-affinity transport system of cyclitols and on the respiration of *Pseudomonas putida* shows that the transport activity is dependent on high energy phosphate bond.

Two systems for active transport of cyclitols have been reported in *Pseudomonas putida*[2]. These systems differ from each other in their affinity for the cyclitol and in sensitivity to osmotic shock. In this report the nature of the energy sources linked to the high affinity, binding protein-dependent, transport system is described. *P. putida* mutants defective in the electron transport chain or in Mg^{++}-Ca^{++} ATPase were not available. Therefore correlation of the transport activities of the cells with their ATP content and respiration rates in the presence of various inhibitors acting at different sites of metabolism was used to investigate the energy source for transport.

Materials and methods. P. putida strain isolation and identification have been described, as has the transport assay[2]. The organism was grown in mineral medium[2] supplemented with either 1% myo-inositol (induced cells) or 1% D-glucose (non-induced cells). Growth temperature was 20 °C. The cells were washed 3 times with mineral medium and resuspended in the assay medium (unless otherwise indicated, mineral medium). 2-/^{3}H/myo-inositol (10 µCi/µmole) concentration was 25 µM, which corresponds to the saturating concentration of the high-affinity transport system. The inhibitors were added to the cells 10 min prior to the transport assay. Anaerobic assays were performed in sealed penicillin vials under O_2-free nitrogen flushing. Flushing was initiated 15 min before the uptake assays. N_2-flushed substrate solution was added and samples were removed through the caps with Hamilton syringes. Conductivity was measured in a RDM_3 conductimeter (Radiometer, Copenhagen). Respiration measurements were performed with the aid of a Clark electrode (Yellow Spring Inc., Ohio, USA), the cell suspension was flushed for 30 sec with air and oxygen consumption was recorded before and after the addition of 0.25 mM myo-inositol. ATP determination and pH measurement during transport under anaerobic conditions were performed as previously described[3].

Results and discussion. Myo-inositol uptake was measured at various pH values. The experiments were performed in 0.1 M Good's buffer[4] adjusted with Tris-base to avoid the presence of alkali ions. Transport activity has a maximum at pH 6 in Good's Tris media (figure 1). However in H_3PO_4-Tris 0.1 M buffers the activities were always lower and the maximum displaced to pH 7.5 (figure 1); ionic strength does not account for the difference observed, since conductivity was not significantly different. Transport activity was also measured at pH 7.5 in different media: it is maximal in either mineral medium or in 0.1 M Tris-HCl, but it is decreased 60% in 0.1 M Tris-H_3PO_4 and 80% in

The effects of inhibitors on myo-inositol transport, ATP content and respiration. Cells were incubated with the inhibitor for 10 min at room temperature. Aliquots were withdrawn for transport assay in the presence of 25 µM myo-inositol; ATP and respiration rate determinations are described in materials and methods. The results are expressed as the percent of the values obtained with untreated cells.

Additions	Transport activity Uptake 15 sec	Time 3 min	ATP content	Respiration rate Endogenous (no substrate)	After substrate addition
None	100%	100%	100%	100%	100%
DNP 1 mM	17%	6%	6%	105%	75%
NaN_3 10 mM	8%	24%	43%	110%	60%
Arsenate 10 mM	76%	87%	80%	100%	100%
Arsenate 10 mM (in absence of phosphate)	34%	44%	50%	97%	100%
NaF 1 mM	95%	100%	100%	100%	100%
KCN 10 mM	8%	9%	0%	0%	0%
Anaerobiosis	75%	42%	55%	-	-