It has been well known that temperature modifies the rate of diapause incidence and the critical daylength in photoperiodic diapause of insects. The mechanism by which temperature modifies the rate of diapause has been extensively studied by Saunders[5-7]. He suggested that diapause is induced when a sufficient number of light-cycles were experienced before the end of the sensitive period, and further that the required number of light-cycles to induce diapause is temperature-compensated and the length of the sensitive period is temperature-dependent. He accounted for the lower incidence of diapause at higher temperatures by the interaction between the length of the sensitive period and the summation of light-cycles. For example, higher temperature leads to faster development, hence loss of photoperiodic sensitivity occurs sooner, and the incidence of diapause is reduced owing to the smaller number of cycles experienced. On the other hand, the manner in which temperature affects the critical daylength has been unclear, but it can be explained by the interaction between the temperature-compensated mechanism of the summation of light-cycles and the quantitative response to daylengths. Saunders assumed that long-day cycles are also inductive (in the diapause induction sense) if sufficient of them are seen before the end of the sensitive period[7]. In the case where animals quantitatively respond to daylengths, Saunders' idea can be extended thus; a longer photoperiod requires animals to experience a larger number of light-cycles before the end of the sensitive period to induce diapause. Then, the prolonged sensitive period at lower temperature enables them to enter diapause under a longer photoperiod.

1 M.J. Tauber and C.A. Tauber, Nature *244*, 296 (1973).
2 N. Minami and M.T. Kimura, Jap. J. Genet. *55*, 319 (1980).
3 M.T. Kimura and N. Minami, Kontyû *48*, 458 (1980).
4 N. Minami, M.T. Kimura and N. Ichijô, Kontyû *47*, 244 (1979).
5 D.S. Saunders, J. Insect Physiol. *12*, 569 (1966).
6 D.S. Saunders, J. Insect Physiol. *17*, 801 (1971).
7 D.S. Saunders, Insect Clocks. Pergamon Press, Oxford 1976.

Liver ferritin synthesis following chronic alcohol administration to rats: Modulation by propylthiouracil

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Summary. Liver ferritin synthesis was inhibited by 22.3% in rats treated with alcohol (2 g/kg) for 45 days. This inhibition was prevented by simultaneous administration (5 mg/kg) of propylthiouracil during the last 15 days. There was no significant effect on liver ferritin concentration.

Ferritin is the major iron-storage protein of mammalian tissues, found predominantly in the liver, spleen and bone marrow[1,2]. In our continuing studies on the effect of alcohol on liver and liver-produced proteins, we have already reported alterations in liver vitamin A stores[3] and plasma protein synthesis[4], and the effect of propylthiouracil (PTU) on these parameters. Here we report a similar effect of alcohol on liver ferritin synthesis and a similar role of PTU.

Materials and methods. Male Wistar rats in 3 groups of 6 animals each (180–220 g) were fed on a 20% protein diet[3], and had constant access to drinking water. Ethanol (2 g/kg) in 20% concentration in sterile saline was injected daily i.p., in 1 intoxicating dose, for 45 days to rats in groups II and III. Rats in control group I received equicaloric glucose by the same route. This caloric intake worked out to about 15% of the total calories consumed by a rat per day. During the last 15 days of the experiment rats in group III received PTU (5 mg/kg) in 20% ethanol. Food consumption of rats in different groups was identical and food was available until the time of sacrifice.

At the end of 45 days each rats was injected i.p. with 10 μCi (370 kBq) of (1-^{14}C)-glycine (9.3 mCi or 344.1 M Bq/mmole from Bhabha Atomic Research Centre, Bombay) per 100 g b.wt, and 120 min after the tracer injection the hepatic portal vein was exposed under ether anaesthesia. The liver was perfused in situ, via the portal vein, with 15–20 ml of ice-cold saline containing 10 mM glycine to flush out blood. The liver was excised, blotted dry, weighed and frozen for ferritin isolation. Ferritin was separated by using a minor modification of the method of Linder and Munro[5]. Ferritin was initially precipitated from the heat-coagulated homogenate-supernatent by ammonium sulphate and the solubilized precipitate was gel-filtered through Sephacryl S-200, superfine (Pharmacia, Uppsala). The protein content of the purified ferritin was quantitated by Lowry's method[6], taking bovine serum albumin as reference standard, since the extinction coefficient of apoferritin is comparable to that of bovine serum albumin[7]. In a separate recovery experiment rat liver ferritin, isolated and purified as described earlier[8], was labelled with ^{125}I (Radiochemical Centre, Amersham) using the chloramine T method[9], and taken through the entire isolation-purification procedure. The recovery averaged 92%. An aliquot of isolated ferritin was counted for ^{14}C in a Nuclear Chicago (720 series) liquid scintillation spectrometer using the dioxane-based scintillator of Geegeebuoy[10]. All the counts were normalized for 80% efficiency. The incorporation of radioactive precursor into ferritin (cpm/mg) was taken as a measure of synthesis[7].

Results and discussion. As shown in the table there was 22.3% inhibition of ferritin synthesis in rats treated chronically with alcohol. Further, this inhibition was prevented by simultaneous PTU administration. The liver ferritin concentration was, however, not significantly affected.

Liver ferritin concentration and ^{14}C-glycine incorporation

Group	Ferritin Concentration (μg protein/g liver	Specific activity (cpm/mg)
I Control	436 ± 45	2939 ± 365
II Alcohol	417 ± 35	2285 ± 167*
III Alcohol + PTU	468 ± 54	3118 ± 539

*$p < 0.02$, the values are mean ± SD for 6 rats.

These observations are broadly parallel with those made on plasma proteins[4].

It has now been conclusively proved that a major proportion of ferritin synthesis in the liver takes place mainly on free polysomes, whereas the synthesis of export proteins, like plasma proteins, occurs predominantly on ribosomes bound to endoplasmic reticulum[11-13]. Further, it has been indicated that proteins synthesized on bound polysomes may be more susceptible to inhibition by ethanol than those synthesized on free polysomes[14,15]. Our present and previous[4] data indicate that both the types of proteins are equally affected by ethanol.

Very little is known about the effects of chronic alcohol ingestion on absorption and storage of iron, and synthesis of liver ferritin in man. From one report it appears that alcohol may increase iron absorption in normal subjects[16]. In many alcoholics parenchymal iron overload is encountered[17]. The stimulation of ferritin synthesis by iron is a very well recognized phenomenon[11]. From all this one would expect that under the conditions of chronic alcoholism ferritin synthesis would be increased. However, our studies in experimental animals suggest that chronic alcohol ingestion may inhibit ferritin synthesis. Recently studies in guinea-pigs have hinted that an adequate tissue concentration of ascorbic acid may be necessary for the maintenance of a quantitative correlation between tissue iron stores and ferritin[18]. This may be an important point for humans, since vitamin C status has been shown to be affected in alcoholism in humans[19].

At present the mechanism of action of PTU is speculative[3,4]. It is known that the level of reduced glutathione (GSH) is decreased by alcohol[20,21]. One of the recently-suggested mechanisms of PTU action is that the GSH levels are significantly raised in PTU-treated rats[22]. Further, the rate of metabolism of ethanol has been shown to be reduced in alcoholic rats treated with PTU[23]. This has strengthened the concept that the thyroid hormones may play a direct or permissive role in the process leading to liver damage. Our observation on liver ferritin synthesis may be yet another manifestation of the beneficial role of PTU in alcoholism.

1 R.R. Crichton, New Engl. J. Med. *284*, 1413 (1971).
2 A. Aisen and I. Listowski, A. Rev. Biochem. *49*, 373 (1980).
3 G.D. Nadkarni, U.R. Deshpande and D.N. Pahuja, Experientia *35*, 1059 (1979).
4 G.D. Nadkarni, U.R. Deshpande and D.N. Pahuja, Biochem. Med. *22*, 64 (1979).
5 M.C. Linder and H.N. Munro, Analyt. Biochem. *48*, 266 (1972).
6 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
7 A.M. Konijn and C. Hershko, Br. J. Haemat. *37*, 7 (1977).
8 G.D. Nadkarni, O.P.D. Noronha, A.B. Sewatkar, U.R. Deshpande and R.D. Ganatra, J. nucl. Med. *21*, 1177 (1980).
9 W.H. Hunter and F.C. Greenwood, Nature *194*, 495 (1962).
10 P.N. Campbell and J.R. Sargent, in: Techniques in protein biosynthesis, vol. 1, p. 304. Academic Press, New York 1967.
11 H.N. Munro and M.C. Linder, Physiol. Rev. *58*, 317 (1978).
12 S.J. Hicks, J.W. Drysdale and H.N. Munro, Science *164*, 584 (1969).
13 C.M. Redman, J. biol. Chem. *244*, 4308 (1969).
14 M.A. Rothschild, M. Oratz and S.S. Schreiber, Gastroenterology *67*, 1200 (1974).
15 M. Oratz, M.A. Rothschild and S.S. Schreiber, Gastroenterology *74*, 672 (1978).
16 R.W. Charlton, P. Jacobs, H. Seftel and T.H. Bothwell, Br. med. J. *2*, 1427 (1964).
17 E. Rubin, in: Alcohol and abnormal protein biosynthesis, p.313. Ed. M.A. Rothschild, M. Oratz and S.S. Schreiber. Pergamon Press, New York 1975.
18 H.P. Roeser, J.W. Halliday, D.J. Sizemore, A. Nikles and D. Willgoss, Br. J. Haemat. *45*, 457 (1980).
19 J.P. Bonjour, Int. J. Vitam. Nutr. Res. *49*, 434 (1979).
20 O. Strubelt, Biochem. Pharmac. *29*, 1445 (1980).
21 C. Guerri and S. Grisolia, Pharmac. Biochem. Behav. *13*, suppl. 1, 53 (1980).
22 W.G. Linscheer, K.L. Raheja, C. Cho and N.J. Smith, Gastroenterology *78*, 100 (1980).
23 R.S. Britton and Y. Israel, Biochem, Pharmac. *29*, 2951 (1980).

Photoperiod-induced changes in temperature-metabolism curve, shivering threshold and body temperature in the pigeon

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Summary. A short photophase combined with cold-acclimation changed the shivering threshold of the pigeon from 21 to 14 °C. The change of critical temperature was also found in the temperature-metabolism curve. A long photophase both alone and combined with warm-acclimation induced a reduction of body temperature to a lower level (about 2 °C) at all ambient temperatures, ranging from 5 to 40 °C.

Seasonal thermoregulatory adaptation, involving insulation and basal metabolic rate (BMR), has been demonstrated in many bird species[1-4]. This adaptation is also indicated by a decrease in a bird's critical temperature in winter[5-7], the decrease being, for example, 10 °C in the willow ptarmigan[5]. It is assumed that seasonal variations in heat production may reflect mainly variations in the overall insulation of the bird[8]. Rautenberg's[9] results showing that the critical temperature of pigeons did not shift during cold- or warm-acclimation lasting for several months in laboratory conditions suggest that during acclimation the adaptation is not insulative. However, when ambient temperature (Ta) is decreased, the heat production capacity of fasted birds seems to be higher when they are cold-acclimated than when they are warm-acclimated (see Hart[10]).

Until now most acclimation studies have been performed under standard photoperiod conditions, thus eliminating the effect of the length of photophase on birds' metabolic adaptation. Our recent results show that the photoperiod may have a strong influence on birds' temperature regulation[11,12] and tissue monoamine and lipid metabolism[13]. In this paper we report experiments investigating the effect of acclimation combined with a natural length of photoperiod (i.e. short photophase and cold-acclimation, and long