acidic plus neutral solvent extract) in brains from control and experimental rats was not significantly different.

Discussion. The data presented here indicate that experimental hyperphenylalaninemia induced in rats by treatment with PCPA + PHE caused a significant reduction in the DPI and TPI content of brain but no change in the PI content. This reduction in polyphosphoinositide content could occur as a result either of an inhibition of its synthesis or of increased degradation. Since the PI content in brains from experimental rats did not increase it is unlikely that the reduction in TPI and DPI observed in hyperphenylalaninemic rat brain is related to increased degradation of polyphosphoinositides. Since the enzymes which phosphorylate PI are present in myelin[18], and a reduction in brain myelin content occurs in experimental hyperphenylalaninemia[7,8], the reduced levels of DPI and TPI may not only reflect reduction in brain myelin levels but could also be the result of their reduced synthesis due to the reduction of PI phosphorylating enzymes. It has been suggested that interconversion of TPI and DPI is responsible for the permeability changes in the membrane during excitation[17,19]. Another possibility is that changes in the permeability properties of myelin and other membranes in brain, which are caused by a reduction in the portion of the unsaturated fatty acids in brain lipids of animals with experimental hyperphenylalaninemia[8-10] are responsible for the reduction in polyphosphoinositide in the brains of hyperphenylalaninemic rats. Since the myelin deficit and the reduction of unsaturated fatty acids in brain also occurs in genetic PKU[20], our present finding raises the possibility that in genetic PKU the brain polyphosphoinositide levels are altered and that the functional abnormalities in the PKU brain could in part be related to the reduction in brain polyphosphoinositides.

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Effect of 5-thio-D-glucose on testicular lipids of mice

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Summary. Spermatogenesis is reported to be completely inhibited by 5-thio-D-glucose in mice. In an investigation of this inhibition, testicular lipid constituents, namely, total lipids, phospholipids, triacylglycerol, free and total cholesterol, 3-hydroxy-3-methylglutaryl-coenzyme A reductase and NADPH generators like glucose-6-phosphate dehydrogenase, isocitrate dehyrogenase and malic enzyme were estimated in mice fed with 5-thio-D-glucose (33 mg/kg) by gastric intubation for 21 days. Significant increase in cholesteryl ester, glucose-6-phosphate dehydrogenase activity and malic enzyme and a decrease in free cholesterol and phospholipids were observed.

A structural analogue of D-glucose, 5-thio-D-glucose[2,3] (5-TG) inhibits glucose uptake and glycolysis not only in liver, kidneys and diaphragm[4,5] but also in the testes[6]. The feeding of 5-TG at a daily dose of 33 mg/kg for 3 weeks inhibited spermatogenesis completely[7]. Our earlier work showed that oral feeding of 5-TG inhibited spermatogenesis, but not i.p. administration[6]. The widespread distribution and the high levels of the lipids are indicative of their importance in the function of testes. The concentration of lipids is very important for spermatogenesis and steroidogenesis. When there is active spermatogenesis, little lipid is evident in the testis but during periods when spermatogenesis is impaired, lipids in various forms accumulate in the interstitial cells[8,9]. As spermatogenesis is inhibited in the testis of mice treated with 5-TG, it was of interest to investigate the lipid constituents and some enzymes which offer reducing equivalents of $NADP^+$ for lipogenesis.

Materials and methods. Male albino mice weighing 25-30 g were used. The animals were maintained on the stock laboratory diet (Hindustan Lever, India) and water ad libitum. 5-TG and glucose-6-phosphate (di-sodium salt) were obtained from Sigma Chemical Co. (St. Louis, USA); DL-isocitrate (barium salt) from Nutritional Biochemical Corporation, digitonin from BDH Laboratory, England; malic acid from Seelse-Hannover, Germany. All others were A.R. grade chemicals. Saline-treated control and experimental animals treated with 5-TG (33 mg/kg) for 21 days were used. The 21-day feeding by gastric intubation was the minimum period for arresting the spermatogenesis completely[6]. All animals were fasted for 24 h and sacrificed by decapitation. The testes were taken for various estimation. The total lipid was estimated by the method of Folch et al.[10]. The free and total cholesterol were estimated by the method of Schoenheimer and Sperry modified by Venugo-

pala Rao and Ramakrishnan[11]. Triacylglycerol was estimated by the method of Fletcher[12] using triolein as standard. Phospholipids were estimated by the method of Taussky and Shorr[13] after digesting the phospholipids with sulphuric acid[14]. The testes were weighed and homogenized in 0.25 M sucrose solution at 4 °C and centrifuged at 15,000 × g for 5 min at 0 °C in MSE Mistral-2L refrigerated centrifuge. All the enzymes were assayed in the supernatant fraction. The activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) (E.C. 1.1.1.34) was measured by the method of Venugopala Rao and Ramakrishnan[15]. The ratio of the extinctions due to hydroxymethyl-glutaryl-CoA and mevalonate in the tissue gives an indirect assessment of hydroxymethyl-glutaryl-CoA reductase activity[15]. A decrease of the ratio indicates an increase of enzyme activity and vice versa. Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) was estimated by the method of Ells and Kirkman[16]. Malic enzyme (E.C. 1.1.1.40) was estimated by the method of Ochoa[17]. Cytosolic isocitrate dehydrogenase (E.C. 1.1.1.42) was estimated by the method of Ochoa[18]. Total protein content was estimated by the method of Lowry et al.[19]. As the protein content was significantly decreased (30%) in the experimental group, the enzyme activities are expressed per g of wet tissue[30].

Results and discussion. The lipid components of the saline-treated control and 5-TG-treated experimental groups are given in table 1. The total lipid content of the testis was slightly increased in the experimental group, but the increase was not statistically significant. There was no significant change observed in the total cholesterol content of the testis but there was a significant decrease (36%) in the free cholesterol and significant increase (35%) in the cholesteryl ester. The increased cholesteryl ester and decreased free cholesterol in the experimental group may suggest that either the hydrolysis of the cholesteryl ester to free cholesterol is diminished or the synthesis of cholesteryl ester from free cholesterol is enhanced by 5-thio-D-glucose. Since free cholesterol is the precursor for the synthesis of steroids, its decreased concentration may explain the defect in the spermatogenesis which agrees with the results obtained by Johnson et al.[9] in the case of TEM, Bauman et al.[21], Bowen and Radin[22], Bennett et al.[23] in the case of the quaking mouse and Younglai and Chui[24] in the case of the steel mouse. In all the above, the free cholesterol was decreased and cholesteryl ester was increased significantly. There was no appreciable change in the triacylglycerol concentration, but there was a significant decrease (49%) in the phospholipid concentration of the experimental testes. The decreased concentration of phospholipids is also responsible for impaired spermatogenesis as the sperm head has a high content of phospholipids. This agrees with the work of Kar et al. who observed the same effect with busulfan[20].

Among the lipids, cholesterol is an important lipid which is needed for steroidogenesis and consequent spermatogenesis. Hence, the rate-limiting enzyme HMG CoA-reductase and its coenzyme, reduced NADP+ (NADPH), were studied. There was no appreciable change observed in the HMG CoA-reductase activity suggesting no alteration in the cholesterol synthesis. Since the total cholesterol remains unaltered and only cholesteryl ester is increased, there is an increased esterification of cholesterol or a defect in the conversion of ester to free cholesterol. For active lipogenesis, NADPH is required by anaerobic processes outside the mitochondria and is often associated with steroid-producing or other lipogenic tissues[28,29].

Also, steroid (androgen) production depends on the side chain splitting enzyme of cholesterol and on the microsomal hydroxylases which are NADPH-dependent as well as on the Δ^5-3β and 17-β-hydroxy steroid dehydrogenases. The potential generators of NADPH in the testes are glucose-6-phosphate dehydrogenase[25,26] isocitrate dehydrogenase[25,26] and malic enzyme[27].

It was observed from the results (table 2) that glucose-6-phosphate dehydrogenase and malic enzyme were significantly (37%; 73%) increased in testes of 5-TG-treated mice. Our earlier study indicates that glycolysis was decreased[6] and other pathways like the hexosemonophosphate shunt pathway, uronic acid pathway and fructose production were increased[30]. Because the alternate pathways play an increased part in the metabolism of glucose, the enzyme glucose-6-phosphate dehydrogenase is generating more NADPH. Though NADPH production is enhanced by 5-TG, spermatogenesis is actually inhibited in the testes. One explanation is a decrease of phospholipids, as the sperm head needs sufficient amounts of phospholipids[20]. The increase of cholesteryl ester may be secondary to a decrease of phospholipids, as the fatty acids of phospholipids are to be used for esterification of cholesterol with the enzyme lecithin: cholesterol acyl transferase (EC. 2.3.1.43). From our work, it is not clear whether a decrease of free cholesterol would affect the synthesis of steroid hormones since even after a quantitative reduction, some free cholesterol is available. Alternately, a block in the conversion of cholesterol to steroid hormones by 5-TG cannot be ruled out.

Table 1. Effect of 5-thio-D-glucose on testicular lipid constituents

Lipid/g wet tissue	Saline-treated testes	5-thio-D-glucose-treated testes
Total lipids (mg)	11.70± 0.77	14.90± 1.47^{NS}
Free cholesterol (µg)	1931 ±17	1652 ±54*
Cholesterylester (µg)	705 ±47	950 ±38**
Total cholesterol (µg)	2636 ±45	2603 ±19^{NS}
Triacyl glycerol (µmoles)	12.40± 0.47	13.70± 0.84^{NS}
Phospholipids (mg)	4.10± 0.18	2.10± 0.42**

Values are given as the mean of 7 mice ± SE of the mean.
*$p < 0.001$; **$p < 0.01$; NS, not significant.

Table 2. Effect of 5-thio-D-glucose on testicular lipid regulating enzymes

Enzyme activities (per g wet weight at 37 °C)	Saline-treated testes	5-thio-D-glucose-treated testes
HMG CoA-reductase activity (ratio of HMG CoA/mevalonate)[15]	2.24±0.33 (6)	2.17±0.38^{NS} (6)
Glucose-6-phosphate dehydrogenase (I.U.)	1.83±0.13 (12)	2.52±0.06* (12)
Malic enzyme (I.U.)	1.43±0.07 (12)	2.47±0.08* (12)
Isocitrate dehydrogenase (I.U.)	1.41±0.12 (10)	1.43±0.11^{NS} (10)

Values are given as the mean ± SE of the mean. Number of animals is given in brackets.
*$p < 0.001$; NS, not significant.

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Enzymatic characterization of nine endoparasite species of small ermine moths (Yponomeutidae)

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Summary. Eight hymenopterous and 1 dipterous species, all endoparasitic in eggs, larvae, or pupae of small ermine moths *(Yponomeuta)* were investigated for their allozyme variation at 3–29 loci. The mean heterozygosity level of the hymenopterous species is one-third of that of the dipterous species. Zymogram patterns of the parasite larvae do not interfere with those of the host.

Electrophoresis is at present the major technique of biochemical systematics[1]. In field samples of insects, one of its possible sources of error may arise from endoparasite proteins, which can be erroneously interpreted as allelic variations of the host. The general zymogram picture of a developing endoparasitisation is an increase in activity of the parasite-specific bands with a concomitant reduction in activity of those of the host[2,3].

Eight parasites of small ermine moths were investigated allozymically as adults and another as larva; they are briefly discussed below (see also data in Dijkerman[4]). The *Yponomeuta* hosts on which they occur are abbreviated as follows and given in parentheses, with those from which they were analyzed in italics: *Y. cagnagellus,* C; *Y. evonymellus,* E; *Y. mahalebellus,* M; *Y. malinellus,* Ma; *Y. padellus,* P; *Y. plumbellus,* Pl; *Y. rorellus,* R; *Y. vigintipunctatus,* V.

The list of parasites comprises the following species (see the table for generic names and families; – note that L2, L3, etc. below denotes the 2nd, 3rd etc. larval stages). *A. fuscicollis:* attacks eggs (*C,* E, M, Ma, P, *R*). Polyembryonic cleavage from L4 on. On the average 80–90 individuals from 1 egg. *D. armillata:* attacks L2–L3 *(V)* or L3–L4 (C, E, M, Ma, P, R). Larva leaves host after it has spun its cocoon. *I. maculator:* attacks pupae, even just before emergence of the adult moth, sometimes L5 (C, E, *Ma, P*). *M. vittator:* attacks presumably late L4 larvae (C, E, M, Ma, P, Pl, *V*). Hyperparasite, parasitises *Diadegma* species. *P. turionellae:* attacks pupae (C, E, P). Reared on *Galleria mellonella* (Pyralidae) in the laboratory. *T. yponomeutae:* attacks L3–L4 *(V).* Presumably thelytokous, only ♀♀ have been reared in the department during the last 10 years (some ♂♂ known from museum collections). *T. tricarinatus:* as *T. yponomeutae* but bisexual. *T. evonymellae:* gregarious, attacks L4, L5 and sometimes pupae (C, E, M, *Ma,* P, R). *D. hyponomeutae:* attacks L4–L5 (C, *Ma, P*).

Electrophoretic techniques and staining methods are the same as described for *Yponomeuta*[3]. Genetic interpretation of the observed variation is inferential. Available data on levels of enzyme polymorphism in Hymenoptera indicate a reduced intrapopulation variability relative to diploid insects[5–7]. Values presented here fall in the limited range of heterozygosity *(H)* levels of the haplodiploid species known[7] ($\bar{H} = 0.029 \pm 0.023$; range 0.000–0.056). When we disregard those species in which less than 10 genomes were investigated (i.e. *I. maculator* and *M. vittator*) the outcome remains the same ($\bar{H} = 0.030 \pm 0.021$; range 0.000–0.055). This average H level for Hymenoptera is less than one-third that of the only dipteran studied ($H = 0.96$). Evidence for the hypothesis that haplodiploidy reduces variation is, however, very weak[9].

Except for *A. fusciollis,* only electrophoresed parasites were studied, whereas larval patterns may interfere with those of their host. The 4 enzymes (namely α-glycerophosphate, lactate and malate dehydrogenase and phosphoglucose isomerase [*Pgi*]) that occur in gels where *Yponomeuta* larvae, parasitized by *A. fuscicollis,* are electrophoresed are all coded for by the same locus in the larval and adult stage, as samples of adults give bands at the same distance of migration as those of the larvae. This also holds for lactate