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Postnatal metabolic changes in the pups of rats exposed to toddy (palm wine) during pregnancy and lactation

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Female rats were exposed to toddy (palm wine) (24.5 ml kg^{-1} body weight day^{-1}) and ethanol (0.52 ml kg^{-1} body weight day^{-1}) before conception and throughout gestation and lactation. For 21 days pups were nursed by their own mothers, afterwards they were fed normal laboratory feed. Examination of the lipid profile of various tissues of 21 day-old pups revealed marked hyperlipidemia. The tissues of 45 day-old pups showed that even though there was some regression in the lipid accumulation, the status of hyperlipidemia prevailed. The lipogenesis was enhanced on both the days studied as evidenced by the increased activity of the lipogenic enzymes, HMG Co A reductase and incorporation of ^{14}C acetate to lipids. Catabolism of cholesterol to bile acids was decreased. The biochemical alterations produced in the pups by toddy and its equivalent in alcohol were different, showing that the non-alcoholic portion of the toddy interfered with the lipid metabolism.

INTRODUCTION

Chronic ingestion of alcohol affects a variety of organ systems and most markedly the liver (Charls, 1980). Maternal exposure to alcohol leads to fetal alcohol syndrome or FAS (King and Fabro, 1983) which is characterised mainly by abnormalities of the nervous system (Clarren and Smith, 1978). Decreased density of synaptic formation was observed in the fetus and pups on exposure to ethanol during gestation (Tanaka *et al.*, 1991). The degree of damage was dose-dependent (Norton and Kotkoskie, 1991) and it affected the development of the CNS (Norton *et al.*, 1988). Most of the studies during the postnatal development of pups of alcohol-exposed dams were on behavioural problems (Driscoll *et al.*, 1985; Abel and Dintcheff, 1986). Consumption of alcohol leads to hyperlipidemia and the mechanism of its formation was well documented (Jones, 1969). Very few studies were conducted to assess alterations in the lipid metabolism of the young of dams administered ethanol during gestation and lactation. Almost all the research work done in alcohol metabolism was carried out with pure ethyl alcohol. But in reality it is alcoholic beverages that are being consumed. Alcoholic beverages contain many pharmacologically active substances other than ethanol. Toddy, (palm wine) is a popular undistilled alcoholic beverage consumed mainly by the low socio-economic strata of the society in Kerala. It is procured from coconut trees (*Cocos nucifera*). Therefore it was decided to study lipid metabolism of pups of dams exposed to toddy/ethanol during gestation and lactation.

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MATERIALS AND METHODS

Female albino rats (Sprague Dawley Strain) of weight 125 g were randomnly selected and divided into three groups of 6 rats each as:

1. control (Laboratory diet);
2. toddy (24.5 ml kg^{-1} body weight); and
3. ethanol (0.52 ml kg^{-1} body weight).

This dosage takes into consideration that a habitual drinker on average consumes 2 bottles (1250 ml) of toddy per day.

The rats were maintained in laboratory conditions in a light and dark schedule of 12 h duration. They were fed with pelleted diet (Lipton India Ltd) and tap water *ad libitum*. The group II animals were given 12 h-fermented toddy purchased daily from the same Government licenced shop and its alcohol content was estimated to be 2–2.5% (AOAC). Ethanol/toddy was administered by gastric intubation. The alcohol content

of the ethanol group was adjusted to be equivalent to that in the toddy group. Ethanol was diluted with water in the ratio 1:7 and toddy was given without dilution. Control rats were fed with an equicaloric amount of glucose solution. After 15 days of treatment they were allowed to mate with normal males for four days. Pregnancy was detected by microscopical examination of vaginal smear. Alcohol/toddy was administered throughout gestation and lactation. The day of delivery was considered as the first day of lactation. Pups were nursed by their own mothers. On the 21st day of lactation, half of the pups were sacrificed by cervical dislocation. The rest of the animals were allowed to grow. They were fed with laboratory pelleted rat feed during this period. On 45th day they were sacrificed and various tissues and serum were collected into pre-cooled containers and analysed by standard procedures. Tissues were stored at −4°C for the analysis of lipids (Menon and Kurup, 1976) and bile acids (Okishio *et al.*, 1967). Activities of enzymes were analysed immediately after tissue collection. Incorporation of ^{14}C acetate to lipids was studied (Molly *et al.*, 1983) as previously described. The activity of the enzymes glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (Kornberg and Horecker, 1960), Malic enzyme (EC.1.1.1.40) (Severo, 1960) and HMG Co-A reductase (EC.1.1.1.34) (Rao and Ramakrishnan, 1975) were also assayed. Protein in the enzyme extract was determined by the method of Lowry *et al.* (1951). Statistical analysis and comparison between groups were done by Student's *t* test (Bennet and Franklin, 1967).

RESULTS

The dams treated with toddy and ethanol showed a significant decrease in the weekly weight gain, in comparison with the control group. The body weight on the day of delivery showed a significant reduction in the toddy treated group and it continued up to the last day of lactation (Fig. 1). Weights of the pups were also lower in the treated groups. A similar pattern in the weight gain was observed in the pups of all groups. On day 45, a drastic weight reduction was noted in the toddy group (Fig. 2).

There was significant reduction in the number of pups born to the dams fed toddy/ethanol. There was a decrease in the survival of pups when they were fed by mothers milk, but it was not statistically significant. Afterwards there were no differences in the survival rates of the pups in the different groups (Fig. 3).

The cholesterol content in the liver and brain of 21st day pups showed a significant increase in both the treated groups. On the 45th day the cholesterol level came down to normal level in the liver of animals administered toddy. In the kidney the cholesterol content did not show any significant alterations in the treated groups in comparison to their control. In brain of 45th day pups, the total cholesterol showed a significant increase in toddy group, while there was no significant change in the ethanol-treated group (Table 1). There was no significant alteration in the total cholesterol content of the serum. However, HDL-C showed a significant reduction in the toddy/ethanol groups on the 21st and 45th days and LDL+VLDL-C showed a significant increase (Table 3).

The phospholipids showed an increase in the liver, brain and kidney of 21st day pups treated with toddy, but there was no change in animals exposed to ethanol. In 45th day pups, a significant increase in phospholipid was found only in the kidney of the treated groups. The serum phospholipids showed a marked increase in both the treated groups in comparison with controls. However, significant alterations were not observed between 21st and 45th day pups (Table 1).

The triglycerides showed a significant increase in all the pups administered toddy, but in the ethanol group there was no significant alteration except in the brain on the 21st day. The free fatty acids showed significant increases in the toddy treated animals on the 21st and 45th day. In the ethanol-administered rats, an increase in free fatty acids was seen only in the liver. The serum triglycerides did not show any significant change on administration of alcohol/toddy. The free fatty acids showed significant increases in treated groups on both days (21st and 45th) (Table 2).

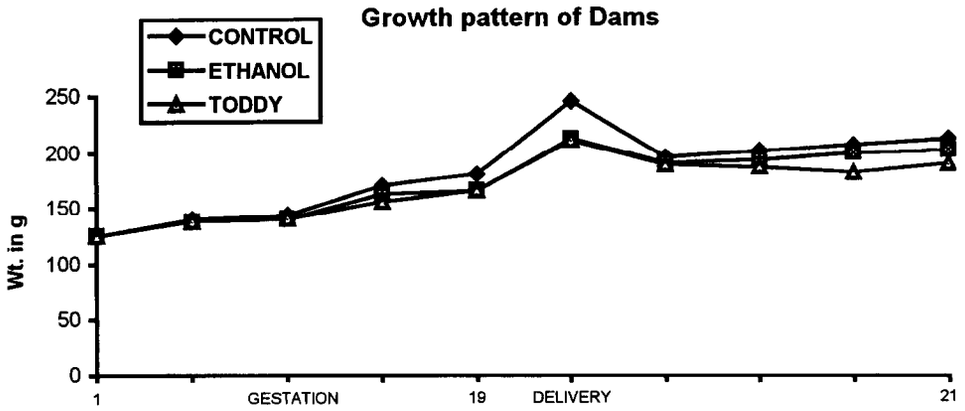

Fig. 1.

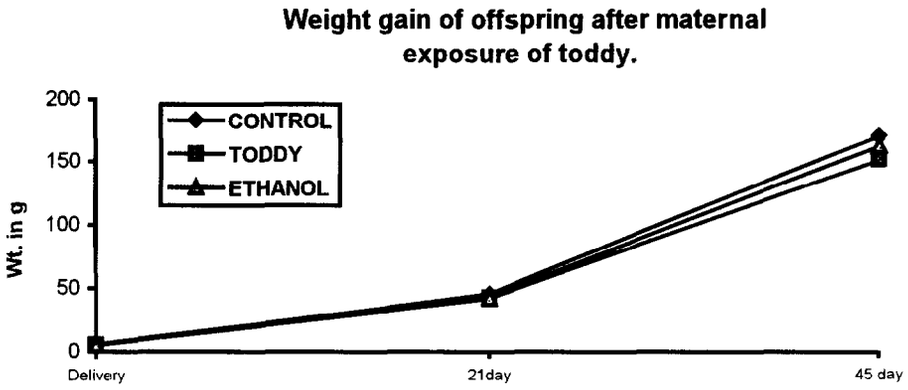

Fig. 2.

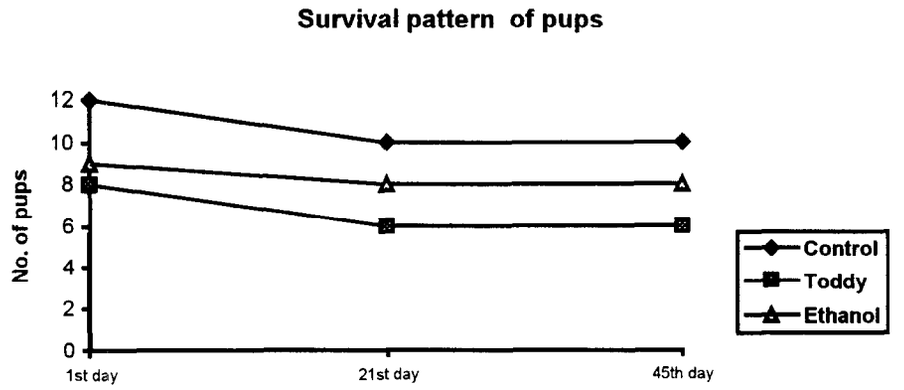

Fig. 3.

Table 1. Lipid profiles of various tissues and serum of offspring

	Liver		Kidney		Brain		Serum	
	21st	45th	21st	45th	21st	45th	21st	45th
Cholesterol content mg $100\,g^{-1}$ tissue								
CON	332 ± 14.8	294 ± 14.4	388 ± 15.4	376 ± 15.6	686 ± 18.8	875 ± 11.4^{b}	80 ± 1.61	83.1 ± 2.50
TOD	420 ± 12.3*	305 ± 10.5^{ab}	377 ± 15.3	369 ± 18.8	890 ± 15.3*	1011 ± 17.9*^{ab}	81.9 ± 2.07	85.7 ± 1.16
ETH	396 ± 13.6*	390 ± 14.3*	375 ± 15.6	352 ± 10.3	866 ± 16.7*	912 ± 19.1^{b}	85.5 ± 1.24	86.1 ± 1.70
Phospholipid content mg $100\,g^{-1}$ tissue								
CON	2042 ± 118.7	2113 ± 112	1943 ± 103	1688 ± 118^{b}	1605 ± 114.6	2074 ± 114^{b}	90.8 ± 4.15	93.8 ± 5.26
TOD	2406 ± 113*^{a}	2129 ± 114^{b}	2411 ± 113*^{a}	1885 ± 117^{b}	1715 ± 112*	2085 ± 118^{b}	141.5 ± 5.2*^{a}	138 ± 5.50*^{a}
ETH	2087 ± 116	2175 ± 119	2085 ± 121	1813 ± 112	1650 ± 119	2014 ± 116^{b}	113 ± 4.86*	114 ± 5.21*

*$p < 0.05$ between control and other groups.
$^{a}p < 0.05$ between group II and group III.
$^{b}p < 0.05$ between 21st day and 45th day. Values expressed as mean $\pm$ SE of six rats on 21st and 45th day.

The total bile acids showed a significant reduction in both the treated groups in 21st and a slight reduction in 45th day pups (Fig. 4). The activities of glucose-6-phosphate dehydrogenase and malic enzyme were elevated significantly in the pups on the 21st day and elevated slightly on 45th day. Maximum activity was observed in toddy-exposed rats. (Fig. 5). The HMG Co-A Reductase activity was high in the toddy- and ethanol-treated groups. The ethanol group showed a slightly higher activity than the toddy group in 45th day pups (Fig. 6).

Incorporation of labelled acetate into lipids showed an increased incorporation into cholesterol, phospholipids and triglycerides in 21st and 45th day pups, but there was a significant decrease in the incorporation to cholesterol ester content (Fig. 7).

DISCUSSION

Administration of toddy/ethanol decreased the weight gain of dams and this is reflected in the weight gain of

Table 2. Lipid profiles of various tissues and serum of offspring on 21st and 45th day

	Liver		Kidney		Brain		Serum	
	21st	45th	21st	45th	21st	45th	21st	45th
Triglyceride content mg $100\,g^{-1}$ tissue								
CON	217 ± 16.8	285 ± 12.6^{b}	231 ± 16.7	299 ± 21.5^{b}	614 ± 18.3	829 ± 18.6^{b}	12.1 ± 1.52	14.8 ± 2.16
TOD	$395 \pm 17.8^{*a}$	$353 \pm 12.9^{*a}$	$263 \pm 16.8^{*}$	324 ± 18.9^{b}	$829 \pm 10.8^{*a}$	$864. \pm 18.5$	12.6 ± 1.28	16.4 ± 2.15
ETH	232 ± 17.9	299 ± 21.8^{b}	231 ± 15.2	319 ± 20.2^{b}	$680 \pm 17.8^{*}$	837 ± 18.9^{b}	12.4 ± 2.10	18.5 ± 2.56
Free fatty acids mg $100\,g^{-1}$ tissue								
CON	331 ± 14.6	421 ± 16.8^{b}	231 ± 28.3	246 ± 10.2	479 ± 14.52	525 ± 12.9^{b}	138 ± 15.1	143 ± 12.2
TOD	$525 \pm 11.0^{*a}$	$635 \pm 12.8^{*ab}$	$288 \pm 16.3^{*a}$	$357 \pm 13.6^{*ab}$	$726 \pm 17.6^{*a}$	$824 \pm 12.5^{*ab}$	$232 \pm 18.2^{*}$	$238 \pm 16.2^{*}$
ETH	424 ± 10.8	$552 \pm 13.7^{*b}$	184 ± 25.6	254 ± 19.5^{b}	498 ± 18.6	543 ± 18.1^{b}	$239 \pm 20.1^{*}$	$248 + 18.9^{*}$

CON, Control; TOD, toddy; ETH, ethanol.
$^{*}p < 0.05$ between control and other groups.
$^{a}p < 0.05$ between group II and group III.
$^{b}p < 0.05$ between 21st day and 45th day. Values expressed as mean ± SE of six rats.

Table 3. HDL-C and LDL + VLDL-C in control and treated groups (mg $100\,ml^{-1}$)

	HDL-Cholesterol		LDL + VLDL cholesterol	
	21st day	45th day	21st day	45th day
Control	28.0 ± 1.04	31.4 ± 2.92	52.0 ± 1.43	51.7 ± 2.87
Toddy	$20.9 \pm 1.63^{*}$	$21.8 \pm 2.44^{*}$	$60.9 \pm 2.86^{*}$	$64.0 \pm 2.94^{*}$
Ethanol	$20.9 \pm 0.88^{*}$	$16.8 \pm 1.11^{*}$	$64.7 \pm 1.42^{*}$	$69.3 \pm 1.47^{*}$

$^{*}p < 0.05$ between control and other groups.

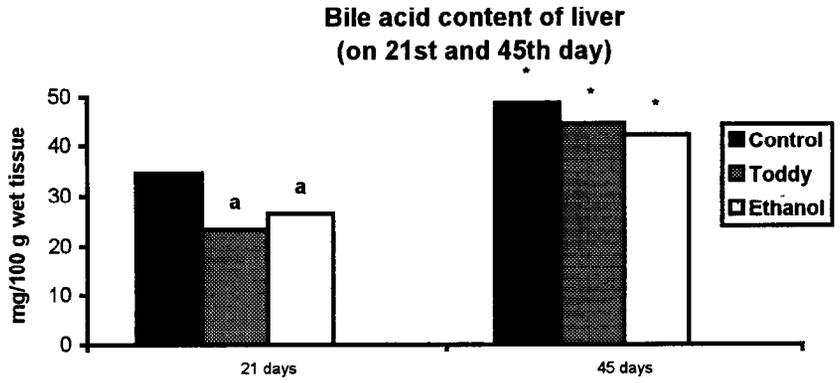

Fig. 4. a = $p < 0.05$ compared to control; * = $p < 0.05$ between 21 and 45 day pups.

pups and in their numbers. This observation is consistent with the earlier reports (Imai and Omoto, 1991; Breese *et al.*, 1993; Maldaner *et al.*, 1994). However, the survival rate of pups during the weanling period and after was not affected. This may be a result of the the low dose of alcohol administration.

In the present investigation, it is observed that ingestion of alcohol/toddy alters all the lipid parameters studied in the pups on the 21st day of lactation. These observations are in agreement with those of Druse (1981) and Lalitha *et al.* (1988), who have reported that cholesterol content of the fetus of alcoholic rats was elevated. Epidemiological studies on alcohol ingestion have shown a strong link between increased serum HDL and alcohol ingestion. Studies on the lipoprotein metabolism in prenatally alcohol-exposed pups are scarce. In this study we have observed low levels of serum HDL levels in 21 day and 45 day pups. This is an alarming factor since HDL is negatively correlated with coronary heart diseases (CHD). This may be a result of increased catabolism of HDL or its decreased biosynthesis. HDL is involved in the trasnsfer of cholesterol from tissues to the liver for degradation. Esterification of cholesterol by labelled studies was found to be decreased indicating lower HDL biosynthesis. Hence, lower HDL levels accords with the observed higher lipid levels in tissues.

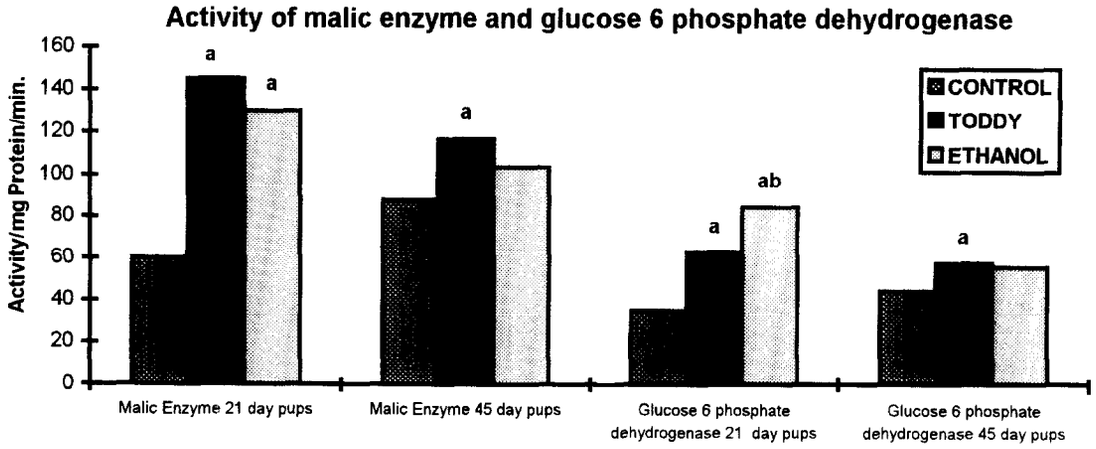

Fig. 5. a = $p < 0.05$ compared to control; b = $p < 0.05$ between treated groups.

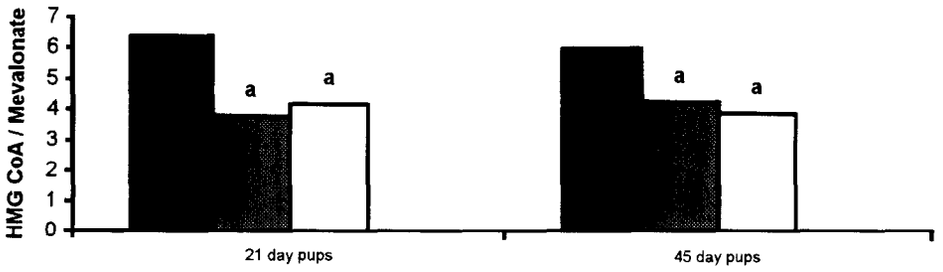

Fig. 6. a = $p < 0.05$ compared to control.

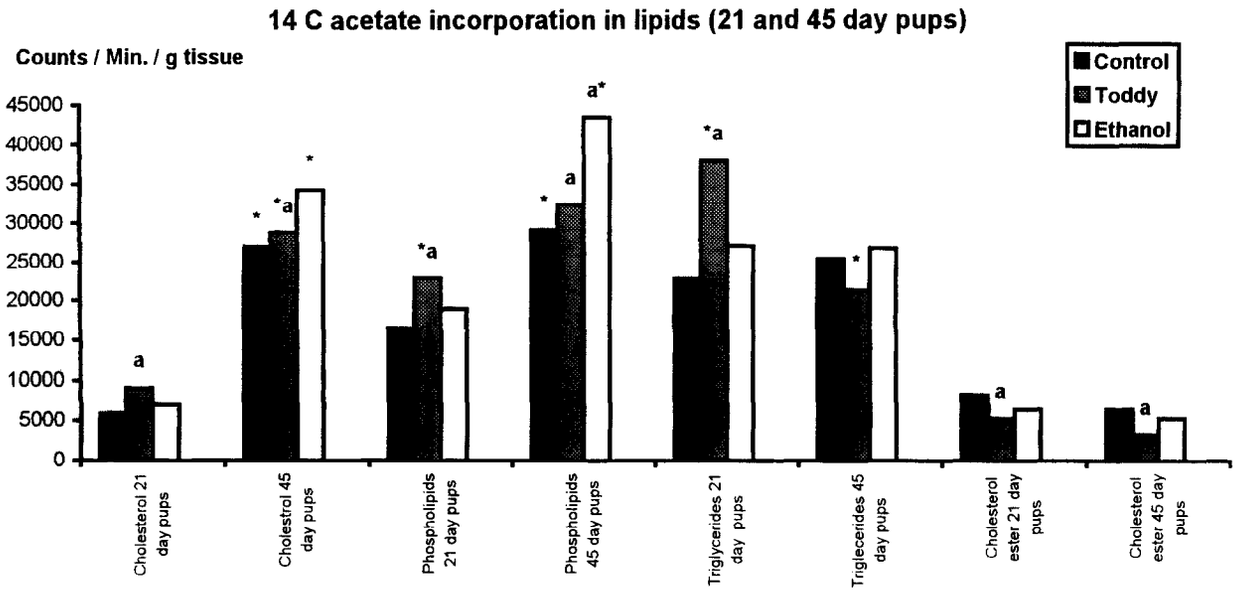

Fig. 7. a = $p < 0.05$ compared to control; * = $p < 0.05$ between 21 and 45 day pups.

Only detailed studies on lipoprotein metabolism will reveal the underlying mechanism.

For 21 days after delivery, the pups were nursed by their own mothers. After that they were fed with a pelleted diet. It is known that milk from the alcoholic rat contains alcohol and its derivatives (Guerri and Sanchis, 1986). Therefore, the enhanced lipogenesis observed may be a result of the alcohol passed on to the pups through milk (Vilaro *et al.*, 1987). The elevation in the cholesterol content is a result of the enhanced lipogenesis, since there is an increase in the activities of lipogenic enzymes such as glucose-6-phosphate dehydrogenase and malic enzyme. The activity of the rate-limiting enzyme of cholesterol biosynthesis—HMG

CoA reductase was also augmented. But the catabolism of cholesterol to bile acids was reduced, since hepatic bile acid content was depleted. This is in agreement with the report of Lakshmanan and Veech (1977) who have observed inhibition of cholesterol 7-alpha hydroxylase by ethanol.

On the 45th day of lactation there was a tendency for the cholesterol and phospholipid level to come down to the control level in the liver, but there were no significant changes in the activities of lipogenic enzymes or bile acid content. This reduction in cholesterol level was the result of a decrease in the synthesis of cholesterol since the activity of HMG Co A reductase was reduced.

In the brain and serum, even on the 45th day, that is when pups are no longer exposed to alcohol, hyperlipidemia is seen. It was shown previously that the peak period of lipid accumulation occurs in the rat brain in the first four weeks of postnatal life (Wells and Dittmer, 1967). So exposure to alcohol during gestation and lactation causes hyperlipidemia in the brain which cannot be reversed by abstaining from alcohol. This altered lipid pattern of the brain may affect the fluidity of membranes and neurotransmission (Lalitha *et al.*, 1988). This can partially explain the various abnormalities associated with FAS.

Analysis of the lipid profile reveals that toddy exerts more drastic changes than ethanol in the tissues. This is caused by increased synthesis and decreased catabolism of lipids in the toddy-exposed pups as evidenced by the activities of lipogenic enzymes, HMG CoA reductase, ^{14}C acetate to lipids and decrease in the hepatic bile acids. The absolute alcohol intake of the toddy group and ethanol group was the same. So the non-alcoholic portion of the toddy is responsible for the augmented lipogenesis exerted by toddy in comparison with ethanol.

Toddy contains 2–5% alcohol (Child, 1972). Toddy contains 15% sucrose (Shamala and Sreekantiah, 1988). Hence, approximately the rats consumed $3.7\,g\,kg^{-1}$ body weight of sucrose day^{-1}. Sucrose is a hyperlipidemic agent (Naismith, 1971). Sucrose feeding was reported to produce higher levels of serum cholesterol when compared with glucose or starch. Reports from our laboratory have shown that sucrose-rich diet caused decreased degradation of cholesterol to bile acids in rats (Molly *et al.*, 1984). In addition to this, Ornoy and Cohen (1980) reported that a sucrose rich diet during pregnancy has teratogenic effects in rats. Jen *et al.* (1991) reported that sucrose reduces newborn weights.

In addition to sucrose, toddy contains B vitamins, ascorbic acid, minerals etc. (Shamala and Sreekantiah, 1988). Various studies have shown that ascorbic acid (Nambisan and Kurup, 1975; Saleena *et al.*, 1985) niacin (Thomas *et al.*, 1982), pyridoxin (Vijayammal and Kurup, 1978) are antiatherogenic vitamins whereas thiamine is atherogenic (Vijayammal and Kurup, 1981). So the concerted action of all the congeners may cause induced marked hyperlipidemia in toddy exposed pups in comparison with alcohol-exposed ones.

It can therefore be concluded that, even though the alcohol content of toddy is very low, because of the presence of unfermented sugars and other congeners it is a potent hyperlipidemic agent. These studies throw light on the fact that pups born and nursed by alcoholic mothers have inborn hyperlipidemia, and it does not recover to normal levels even when the pups are not exposed to alcohol.

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