The Influence of a Low Lipotrope Diet on Response of Maternal and Fetal Rats to Lasiocarpine

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SUMMARY

Lasiocarpine has been administered intragastrically to pregnant rats maintained on control and on lipotrope-deficient diets during gestation. Effects of the toxin on the maternal liver have been noted as well as the transplacental effects on the newborn rat at birth and at seven weeks postpartum. Exposure to the toxin in utero resulted in significant liver lesions in seven-week-old rats with most severe changes occurring in offspring littered to females fed the low lipotrope diet. Exposure to the toxin in utero resulted in significant liver lesions in seven-week-old rats with most severe changes observed in offspring littered to females fed the low lipotrope diet.

INTRODUCTION

The pyrrolizidine alkaloids occur in many species of three important plant families, including the genera Senecio, Crotalaria, and Heliotropium, and they constitute a hazard to man and animals in many parts of the world. Sheep, cattle, and horses develop a slowly progressive fatal liver disease after ingesting the toxic plants (2, 3). Cirrhosis of the liver in horses as a result of feeding on ragwort (Senecio jacobea) was reported as early as 1903 (2), and according to Campbell (4) this was the first indication that this plant was hepatotoxic. Reports from widely separated areas of the world, including New Zealand (Winton disease), Germany (Schweinsberger's disease), Nova Scotia (Pictou disease), Norway (Sirszyke), Nebraska (Walking disease), and South Africa (Molten disease or Dunsiekte) have confirmed the extensive distribution of liver disease resulting from ingestion of various species of Senecio (9).

Contamination of edible flour with Senecio species has been associated with human liver disease in Cape Province and in Cape Town (17). Potentially dangerous consequences may also occur from the administration of the alkaloids in medicinal preparations (13), and a disease in children has been associated with consumption of "bush tea" contaminated with Senecio alkaloids in the West Indies and in South Africa (1, 9). The common occurrence in man of cirrhosis and primary liver cancer in areas where Senecio is known to be ingested has suggested an etiologic association which thus far is unproven.

A chronic, progressive liver disease has been induced in rats by administration of a single dose of a pyrrolizidine alkaloid (14). It is significant that the toxicity of the alkaloid was accelerated when dietary protein was restricted. In addition, malignant liver cell tumors have been induced in rats with moderate, intermittent doses of pyrrolizidine alkaloids (15) and Campbell (4) has produced tumors in fowls by administering an alkaloid extracted from Senecio jacobea (seneciphylline).

The preceding observations point to a direct action by the Senecio alkaloids on the liver parenchymal cells and illustrate the potential carcinogenic hazards to man and animals. We have been interested for a number of years in the relationship of dietary factors to the induction of liver cirrhosis and carcinoma. This paper reports the results of investigations in which pregnant and lactating female rats were fed control diets or diets low in lipotropes and exposed to lasiocarpine.

MATERIALS AND METHODS

Female rats of the Charles River caesarean-derived strain (Charles River Breeding Laboratories, North Wilmington, Massachusetts) were fed their respective diets beginning on Day one of pregnancy, based on positive vaginal swabs, and continued on diet through lactation. Composition of the diets used in the experiments is shown in Table 1. The peanut meal used in the diet was alcohol extracted and shown to be free of aflatoxin by chemical assay. Single, screen-bottom cages were used to house the pregnant females; feed and water were supplied ad libitum. Lasiocarpine solutions3 as the sulfate were freshly prepared.

Dosing was by gastric intubation. Some females were given a single dose of lasiocarpine on Day 13 and were killed on Day 20. Others were dosed on Day 14 and killed on Day 21, and still others were dosed on two gestational days (13 and 17) and killed on Day 20. Some females from the various groups were allowed to litter normally and, where possible, some of the offspring were continued on diet to weaning and beyond in order to observe later postnatal effects. Gross observations were made, placental and fetal weights were recorded, and tissues were taken from all specimens and routinely processed for histologic study.

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3Chemicals were supplied by Dr. C. C. J. Culvenor, Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia.
Table 1

<table>
<thead>
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<th>Ingredients</th>
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<td>Peanut meal (Alcoholic extract)</td>
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Composition of the diets used.

RESULTS

Tables 2 and 3 list results of administration of 100 mg/kg of lasiocarpine to rats on Day 13 of gestation. Five of eight females fed the control diet and dosed with the alkaloid on Day 13 succumbed, three of them on Day 17 and one each on Days 18 and 19. Some of these females were examined shortly after death, and most fetuses had died prior to the demise of the mother. Of the three females surviving, one was killed on Day 20 with all fetuses alive, two were killed on Day 22 with one entire litter dead, and the other with both live and dead fetuses present in the uterus.

Eight females were fed the low lipotrope diet and dosed with lasiocarpine. One of the eight had no evidence of pregnancy, four of the eight succumbed on consecutive Days 17–20, two had resorbed the conceptus, and two had dead fetuses in utero. The animal killed on Day 20 had resorbed, one of the two killed on Day 22 had both live and dead fetuses, and the other had resorbed.

Lasiocarpine administered to mother rats fed the control or low lipotrope diet caused maternal liver necrosis which was more severe in the animals fed the low lipotrope diet (Figs. 1, 2). Some lasiocarpine-treated females had medial hypertrophy of the pulmonary arteries (Fig. 3) irrespective of dietary regimen, and large, bizarre cells were observed in the lungs of many of them (Fig. 4). These changes appeared to have been a bit more numerous in the low lipotrope-treated rats, but no effort was made to quantitate them. Fetal and placental weights were decreased on both diets as a result of the exposure to lasiocarpine (Table 3). The total number of fetuses was reduced in the low lipotrope-treated groups. Two of twenty-four were dead in control-treated, while 3/10 were dead in the low lipotrope-treated groups, the former representing two litters and the latter representing a single litter. There was a higher incidence of liver necrosis in the low lipotrope groups (5/13) compared to control-treated fetuses (1/24). Acute liver necrosis and hemorrhage were characteristic lesions observed in most fetuses in which lesions were observed, but some exhibited only bland, parenchymal cell degeneration. Lipid accumulation was usually present in the liver of fetuses born by dams fed the low lipotrope diet (Figs. 5–8).

Tables 4 and 5 list results from the series in which female rats were fed the control and low lipotrope diets, dosed with 75 mg/kg body weight, and killed on Day 21. Three of eight females fed the control diet and dosed with lasiocarpine aborted, 1/8 died on Day 20, and 4/8 had both live and dead fetuses (Tables 4–5) when killed on Day 21. Three of eight females fed the low lipotrope diet and dosed with lasiocarpine died, two on Day 20 and one on Day 21. The fetuses of all three of these had died prior to the demise of the mother. One of eight aborted, one was not pregnant, and 3/8 had both live and dead fetuses when killed on Day 21.

Lasiocarpine resulted in smaller fetuses (Table 5) with more severe reduction in weight in those whose dams were fed the low lipotrope diet. Placenta weight was decreased about equally in both groups. Characteristic necrosis and hemorrhage were observed in the livers of all control-treated females, in 6/8 of low lipotrope-treated females, in 21/41 fetuses whose mothers were fed the control diet and dosed with lasiocarpine, and in 19/33 fetuses exposed to the low lipotrope diet and lasiocarpine. Females of both groups had the previously described large cells in the lungs. In general, the effects of this level of treatment were more marked than those observed at the higher level of 100 mg/kg body weight, perhaps a result of the better survival of mothers and fetuses in this group which permitted a more profound response to treatment. The further enhancing effect of the low lipotrope diet was also more pronounced in this experiment.

A third series of pregnant rats was exposed to lasiocarpine, 70 mg/kg body weight, in divided doses of 35 mg/kg each on gestational Days 13 and 17. One control and two control-treated females were killed on Day 20 to observe the effects of this level of lasiocarpine on near-term fetuses (Tables 6, 7). Neither of the treated females had liver necrosis (Table 6), but the fetal livers exhibited characteristic lasiocarpine damage (Figs. 9, 10). One control-treated and two low lipotrope-treated females died two to four days postpartum, and the remainder were carried on experiment until the young were weaned. The treated-control female, dying four days postpartum, had liver necrosis, and 1/5 killed 30 days postpartum had liver damage, but the remaining four had morphologically normal livers except for an occasional enlarged liver cell nucleus. One low lipotrope-treated female, killed on Day 20 had liver necrosis, as did both of those succumbing at two and four days postpartum. Liver necrosis was observed in some of the offspring from these litters. However, one low lipotrope-treated female had no liver necrosis while her offspring did (Figs. 11, 12). Compared to the two higher dose experiments, both the females and the fetuses on this experiment were considerably less affected by lasiocarpine regardless of the dietary treatment. In contrast to the two prior experiments, most females in the third experiment gained weight during pregnancy. In the two prior experiments, most treated females had a net loss of weight following exposure to lasiocarpine. There were fewer dead or stillborn fetuses and the fetal and placental weights were decreased only in the low lipotrope-treated group. Fetal liver necrosis did not differ significantly between the two groups with 3/19 and 3/20 on control and lipotrope diets respectively having microscopic evidence of liver damage. It is significant that in some of the females a dose schedule on two gestational days resulted in no detectable maternal liver damage over and above that induced by diet, but fetal liver deterioration was initiated in utero.
Table 2

<table>
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<th>Diet</th>
<th>Female rat No.</th>
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Effect of low lipotrope diet and lasiocarpine on pregnant rats treated on Day 13.

Table 3

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<th>Diet</th>
<th>Lasiocarpine</th>
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<th>Mortality</th>
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Effect of low lipotrope diet and lasiocarpine, 100 mg/kg body weight on Day 13 of gestation (died prior to or were killed on Day 20 or Day 22).

Surviving offspring from this experiment have been kept on their respective diets for long-term studies and will be reported at a later date. A few from each group were killed seven weeks after birth and some of these had interesting liver lesions. In the control dietary groups exposed in utero, there was bile duct proliferation and enlargement of parenchymal cell nuclei in the periportal zone (Fig. 13) and enlargement of nuclei in the centrilobular zone (Fig. 14). Similar but more severe changes were seen in the periportal area of the low lipotrope-treated animals (Fig. 15). Toward the centrilobular zone, megalocytes and hyperplasia of the parenchymal cells were observed (Fig. 16).
Table 4

<table>
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<th>Diet</th>
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Effect of low lipotrope diet and lasiocarpine on pregnant rats treated on Day 14.
* Dead on this gestational day.

Table 5

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<th>Lasiocarpine</th>
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<th>Litter</th>
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</table>

Effect of low lipotrope diet and lasiocarpine, 75 mg/kg body weight on Day 14 of gestation (killed on Day 21).

DISCUSSION

Many plants have a well-known poisonous effect on the liver and would not be included in the human diet under ordinary circumstances. Cirrhosis and liver cancer have been prevalent in many areas of the world for a long time and speculations about their etiology have covered a broad range of factors including infectious diseases, parasites, and malnutrition. In recent years, however, there has been some doubt that these factors alone could account for the incidence of the diseases so
Table 6

<table>
<thead>
<tr>
<th>Diet</th>
<th>Rat No.</th>
<th>Lasiocarpine (mg/kg)</th>
<th>Wt. change (gm)</th>
<th>Liver Fat</th>
<th>Necrosis</th>
<th>Other</th>
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<td>Control</td>
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<td>+123</td>
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<td>Killed at Day 20</td>
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<tr>
<td></td>
<td>756</td>
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<td>+130</td>
<td>0</td>
<td>0</td>
<td>Killed 1 month, postpartum</td>
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<tr>
<td></td>
<td>757</td>
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<td>+142</td>
<td>0</td>
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<td>758</td>
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<td>Killed 1 month, postpartum</td>
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<tr>
<td></td>
<td>759</td>
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<td>+93</td>
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<td>760</td>
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<td></td>
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<td>763</td>
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<td>764</td>
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</tr>
<tr>
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<td>2+</td>
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</tr>
<tr>
<td></td>
<td>772</td>
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<td>+64</td>
<td>3+</td>
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<tr>
<td></td>
<td>773</td>
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<tr>
<td></td>
<td>774</td>
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<tr>
<td></td>
<td>776</td>
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<td>+50</td>
<td>3+</td>
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<td>Died 2 days postpartum</td>
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<td></td>
<td>777</td>
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<td>+24</td>
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<td>0</td>
<td>Killed 1 month, postpartum</td>
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<tr>
<td></td>
<td>778</td>
<td>70</td>
<td>+34</td>
<td>2+</td>
<td>1+</td>
<td>Died 4 days postpartum</td>
</tr>
</tbody>
</table>

Effect of low lipotrope diet and 2 doses of lasiocarpine, 35 mg/kg each, on gestational Days 13 and 17.

prevalent in many countries. Until recently, little attention has been paid to toxins of plant or fungal origin, alone or in combination with dietary inadequacies, as etiologic agents in human disease. The surge of interest in mycotoxins occasioned by the discovery that mold toxins can represent a serious potential hazard to man and animals (19) resulted from outbreaks of disease in animals. These epizootics have awakened plant and animal scientists to the need for research in this important area of toxicology.

The realization that the Senecio alkaloids, in addition to their practical importance as an economic problem in livestock, may be causative factors of some human liver diseases has resulted in renewed interest in this group of hepatotoxins. Some of the alkaloids may be suitable for the study of the mechanism of the evolution of tumors in experimental animal models. However, before such studies can be conducted, it must be confirmed in more than one laboratory and with several strains of rats that the alkaloids are indeed carcinogenic. The profound delayed impact of the action of lasiocarpine and other members of the group on the liver indicates the need for an energetic attack on the problem of the mechanism of liver damage whether or not the toxins are carcinogenic. Since the initial action may take place in the early stages of life in the rat, it seems likely that infantile liver disease might result from contamination of human or cow's milk. This hypothesis is supported by the interesting observation of Schoental (13) that the administration of lasiocarpine to lactating rats, in doses having no apparent effects on the mothers, caused fatal liver lesions in the young. We have now shown that in utero exposure to lasiocarpine can cause fetal liver damage without appreciable maternal liver injury.

Spatz and Laqueur (18) have induced tumors in rats by transplacental exposure to crude cycad material, and Carnaghan (5) has shown that a single dose of aflatoxin to rats early in life can cause liver cancer that appears two years or more after exposure to the carcinogen. Experiments with pregnant rats indicate that aflatoxin does not result in liver damage to the rat fetus (our own laboratory, unpublished data) but this question needs further investigation since Ellis and DiPaolo (7) have demonstrated a positive teratogenic effect in hamster embryos. The teratogenic effects of heliotrine reported by Greene and Christie (8) have been referred to previously.

Dietary factors cannot be ignored in the study of carcinogenesis any more than viral infections and parasites. The induction of nutritional cirrhosis potentiates the liver of rats to the carcinogenic action of aflatoxin (11) and diets low in lipotropic factors result in a shorter induction time and a higher incidence of tumors associated with aflatoxin (12). Population groups, particularly in technologically developing countries, often consume diets which are low in protein and limiting in sulfur-containing amino acids. Evidence is accumulating to show that in many areas of the world those who are subject to dietary deficiencies are also exposed to dietary contaminants as well as hepatotropic viral infections and parasites; these factors must be considered in epidemiologic surveys as well as in basic research aimed at the elucidation of the nature and causes of liver disease and cancer (10, 12, 19).

The results of studies reported in this presentation indicate that the maternal diet can appreciably alter the effect of a toxin on the developing embryo and can result in an enhancement of liver changes several weeks after birth. This
observation parallels, in part, the findings of Schoental and Magee (16) in which a diet low in protein accelerated the toxic effect of a pyrrolizidine alkaloid administered to rats.

The pyrrolizidine alkaloids have been shown to have powerful mutagenic action in the fruit fly Drosophila melanogaster (6), and Greene and Christie (8) have induced malformations in rats by dosing the mother during various stages of pregnancy. The natural occurrence of these mutagens in plants presents many questions relative to their continuous action after ingestion. Those not fatally affected by the hepatotoxic action might sustain genetic deterioration which could manifest itself at a much later time when prior exposure would be unknown or unsuspected.

Consideration of the hepatotoxic effects of lasiocarpine and the other Senecio alkaloids, as well as their carcinogenic potential and the enhancing effects of dietary modifications, makes these substances important tools in the study of liver disease and possibly in cancer. It appears extremely important to confirm the carcinogenic potential of the major alkaloids, to determine the effects of cofactors such as dietary treatment and other known liver toxins or carcinogens on potentiation of the action of the alkaloids, and to better define similar and dissimilar biochemical and morphologic effects of toxins and carcinogens. Such studies are in progress in our laboratories.

**REFERENCES**


**Table 7**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Lasiocarpine</th>
<th>Average wt. change (gm)</th>
<th>Mortality</th>
<th>Liver Fat</th>
<th>Necrosis</th>
<th>Litter</th>
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<td>Maternal</td>
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<td></td>
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<tr>
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<td>0/4</td>
<td>4/4 Live</td>
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<td>+62</td>
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<td>2/8 Live</td>
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<td>0/4</td>
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<td>0/4 Live</td>
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<td>+50</td>
<td>2/8</td>
<td>8/8</td>
<td>3/8 Live and dead</td>
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Fetal

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<th>Average wt. change (gm)</th>
<th>Average placenta wt. (gm)</th>
<th>Liver necrosis</th>
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<tbody>
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<td>27</td>
<td>3.7</td>
<td>0.54</td>
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<td>29</td>
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<td>3.6</td>
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<td>+</td>
<td>24</td>
<td>2.5</td>
<td>0.48</td>
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</table>

Effect of low lipotrope diet and lasiocarpine, 2 X 35 mg/kg, Days 13 and 17 of gestation.

*Some kept on experiment.
Fig. 1. Photograph of liver section from a female fed the control diet, dosed with lasiocarpine 100 mg/kg body weight on Day 13, and killed on Day 20. Widespread necrosis and hemorrhage were present in all lobes of the liver. H & E, × 140.

Fig. 2. Photograph of liver section from a female fed the low lipotrope diet, dosed with lasiocarpine 100 mg/kg body weight on Day 13, and killed on Day 20. Characteristic hemorrhagic necrosis was present in all lobes of liver with even more widespread distribution than that observed in dosed, control females. Lipid accumulation can be observed in lower left portion of photograph. H & E, × 140.

Fig. 3. Pulmonary artery from a female fed the control diet and dosed with lasiocarpine. Medial hypertrophy was observed in some animals from each group irrespective of dietary treatment but venoocclusive lesions of the liver were not observed in any of the animals. H & E, × 400.

Fig. 4. Characteristic large, bizarre cells (arrows) seen in lungs of a number of the females dosed with lasiocarpine. Dietary treatment did not appear to be related. H & E, × 400.

Fig. 5. Liver section from fetus taken on Day 21 from a female fed the control diet. Parenchymal cells are normal for this stage of development. H & E, × 400.

Fig. 6. Liver section of fetus taken on Day 21 from a female fed the control diet and dosed with lasiocarpine 75 mg/kg body weight on Day 14; lesion primarily necrosis. H & E, × 140.

Fig. 7. Higher magnification of an area from Fig. 6 illustrating parenchymal cell necrosis. Kupffer cells are still visible, and many sinusoids are intact at this point with lesions in situ. Later, breakdown occurred resulting in large confluent areas of necrosis. H & E, × 400.

Fig. 8. Liver section of fetus taken on Day 21 from female fed low lipotrope diet and dosed with 75 mg/kg body weight on Day 14. Lipid droplets and severe hemorrhagic necrosis are present. Compare to Fig. 7. H & E, × 400.

Fig. 9. Photograph of a liver section from a female fed the control diet and dosed with lasiocarpine, 35 mg/kg body weight on Days 13 and 17 of gestation and killed on Day 20. Liver necrosis is not present, but a small amount of hydropic change is evident. H & E, × 400.

Fig. 10. Photograph of liver section taken from the fetus born to the female referred to in Fig. 9. Parenchymal necrosis is similar to that observed in offspring from control-treated females with maternal liver lesions. H & E, × 400.

Fig. 11. Photograph of liver section from a female fed the low lipotrope diet and treated otherwise as the female referred to in Fig. 9. The characteristic large droplet lipid accumulation is the only morphologic evidence of injury H & E, × 400.

Fig. 12. Section of liver from the fetus born to the female of Fig. 11. Although dam had no evidence of liver injury from lasiocarpine, fetal liver necrosis and hemorrhage was widespread. H & E, × 400.

Fig. 13. Periportal bile duct proliferation, parenchymal cell necrosis, and nuclear enlargement in seven-week-old offspring from a female fed the control diet and dosed with lasiocarpine, 35 mg/kg body weight on Days 13 and 17 of gestation. H & E, × 400.

Fig. 14. Centrilobular area from liver of rat in Fig. 13. There is evidence of slight parenchymal damage and nuclear enlargement. H & E, × 400.

Fig. 15. Liver section from seven-week-old rat littered to a female fed the low lipotrope diet and dosed with lasiocarpine 35 mg/kg body weight on Days 13 and 17 of gestation. Parenchymal cell necrosis, bile duct proliferation, and megalocytosis is present in periportal zone. H & E, × 400.

Fig. 16. Centrilobular zone of liver of rat in Fig. 15. Slight lipid accumulation with megalocytosis and early focal regeneration of the parenchyma are primary features of this area of the liver. Such areas were commonly observed in some of the rats killed at the seven-week postpartum period. H & E, × 400.
Diet and Lasiocarpine Effects

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The Influence of a Low Lipotrope Diet on Response of Maternal and Fetal Rats to Lasiocarpine

P. M. Newberne


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