Summary. By the techniques of zygote transfer and allotype suppression, b^5b^5 homozygous rabbits were produced with an altered expression of immunoglobulin light chain allotypes; i.e., at 24 weeks of age, only 27% of the total γG was b5γG compared to 90% in nonsuppressed homozygotes. Of the 73% b-negative molecules, 44% were c7γG and 29% were both b- and c-negative. These results suggest that there may be at least three "loci" which control the synthesis of light chains.

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Hepatic Lipidosis Associated with L-Asparaginase Treatment* (33644)

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Although fatty livers have long been recognized, it is only comparatively recently that the factors related to the deposition of fat in this organ have been understood. This condition has been referred to as a fatty degeneration, a fatty metamorphosis, and as a fatty infiltration. According to most viewpoints, fatty infiltration results from an abnormal accumulation in this organ of lipids, predominantly triglycerides, which have been transported there from other tissues.

In late March of 1967 a biopsy specimen was obtained from patient F. H., a terminal acute lymphatic leukemia patient who had received some 200,000 units of *E. coli* L-asparaginase over an interval of 32 days, during which time he had shown a dramatic response and excellent remission (1). Light microscopy of the specimen revealed what appeared to be fatty infiltration of the liver. These results of fatty metamorphosis of the liver associated with L-asparaginase therapy were reported to the leukemia chemotherapeutic task force by Dr. J. M. Hill of this institute in May of 1967. These findings served as the starting point of this investigation.

The production of fatty liver by deficiency (2) or imbalance (3) of amino acids has long been recognized. Many other agents, ethionine (4), orotic acid (5), tetracycline (6), aureomycine (7), ethanol (8), carbon tetrachloride (9), and bacterial endotoxins (10), can also produce fatty liver. However, the production of fatty liver by L-asparaginase, an enzyme, has only recently been suggested. The present studies show that low specific activity L-asparaginase induces a fatty liver in normal male C3H mice and that the accumulated lipids are essentially all triglycerides. On the other hand, enzyme of rather high specific activity does not show the same effects.

Materials and Methods. Liver tissues obtained at autopsy of several L-asparaginase-treated patients were suspended in physiolog-

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ic saline solution, cut into fragments, and all excess blood removed by saline washing. They were blotted free of excess saline solution, frozen, lyophilized, and stored at 4° under nitrogen until processed.

Normal male C3H mice were employed as experimental animals to test the steatogenic potency of various preparations of *E. coli* L-asparaginase kindly supplied by Drs. J. M. Hill and Joseph Roberts of this institute (11). It should be emphasized that these enzyme preparations represented the best products then available to us, but very likely, even the purest preparation was only 50–60% L-asparaginase. Three animals per treatment group received i.p. injections of L-asparaginase of variable purity, ranging in dose from 100 to 1000 iu/kg/day, repeated on 2, 5, or 10 consecutive days. Control animals received 0.1 ml of physiologic saline for the same period.

The day following the last therapeutic injection, the animals were sacrificed, the livers were excised promptly, minced, and freeze-dried. Weighed specimens of the dry tissue were analyzed for total lipids by repeated extractions with chloroform–methanl, 2:1 (v/v), and these combined lipid extracts were partitioned according to Folch *et al.* (12). The organic phase was dried with anhyd. Na_2SO_4 and evaporated *in vacuo* at low temperature. A comparison of the weight of the resultant lipid with the weight of the tissue from which it was obtained permitted a calculation of the percentage of total crude lipids on a dry weight basis.

Aliquots of the total crude lipids were then analyzed by thin-layer chromatography (TLC) utilizing silica gel G and two solvent systems. The first, consisting of petroleum ether, diethyl ether, and acetic acid (90:10:1) was employed for the fractination of the nonpolar lipids, and the second, consisting of chloroform; methanol; ammonium hydroxide; water (75:25:1:4), was utilized to separate the more polar lipids. Authentic standards in known amounts were included on each chromatographic plate for identification and subsequent quantitation. After development, the plates were sprayed with 50% H_2SO_4 and charred at 180°. Quantitative densitometry of the unknown charred spots by comparison with those of the authentic standards allowed for identification as well as for quantitation. During this investigation, 10 groups (3 mice/group) of control animals were included for comparative purposes. It was found that the analyses of their total liver lipids and of percentage of triglycerides had a coefficient of variance of 12%.

TABLE I. Steatogenic Potency of Partially-Purified L-Asparaginase.

	% Crude lipids (dry wt.)	TG[a]	PL[a]
Control (physiological saline)	15.8	14.3	82.0
Group I (200 IU/kg × 5)	25.8	25.2	73.0
Group II (1000 IU/kg × 5)	22.4	31.6	64.0

[a] Percentage of crude liver lipids.

Gas-liquid chromatography of liver lipid fatty acid methyl esters was employed as an adjunct to this investigation. Also, sections of experimental and control animal livers were studied using light and electron microscopy.

Results. Effect of low specific activity L-asparaginase injection on the concentration of mice liver lipids. Table I shows the change in mouse liver lipids after the injection of low specific activity enzyme (55 iu/mg of protein) on 5 consecutive days. The level of triglycerides (TG) increased and the amount of phospholipids (PL) decreased proportionately. This accumulation of lipids was confirmed by histologic examination.

Results on the accumulation of lipids in mouse liver after the administration of various doses on varying dose schedules of L-asparaginase (55 iu/mg of protein) are shown in Table II. As shown, only in Group II is there a significant fatty infiltration. However, in Group I there is a suggestion, judged from free fatty (FFA) as well as TG, that even this brief period of L-asparaginase treatment can induce fatty infiltration.

Effect of high specific activity L-asparaginase injection on the concentration of mice liver lipids. Table III shows the quantitative

TABLE II. Murine Hepatic Lipidosis Following Administration of Partially-Purified L-Asparaginase.

	% Crude lipids (dry wt.)	TG[a]	FFA[a]
Control (physiological saline)	13.9	9.8	5.5
Group I (200 IU/kg × 2)	14.8	13.4	11.1
Group II (200 IU/kg × 5)	24.9	19.2	4.0

[a] Percentage of crude liver lipids.

determinations of total lipids and triglycerides after the injection of high specific activity enzyme. It is seen in Groups I and II that enzyme of high specific activity (250 iu/mg of protein) does not exhibit significant steatogenic potency in mice. Table IV demonstrates that other preparations of enzyme of high specific activity (300 iu/mg of protein) also had negligible steatogenic potency.

The fatty acid composition of mouse liver lipids. The fatty acid composition of mouse liver lipids following the administration of *E. coli* L-asparaginase was analyzed by gas-liquid chromatography. The only noticeable changes were measurable amounts (5–10%) of palmitoleic acid (16:1) seen in enzyme-treated animals compared to trace quantities in control animals.

Discussion. It is well established that fatty livers result from the accumulation of essentially one class of lipids, triglycerides. Lombardi and Oler (13) reported that this lipid normally represents only 5–10% of rat liver total lipids. In this investigation determination of both total crude lipids and triglyceride concentration proved most reliable for detection of hepatic lipidosis.

Rakieten *et al.* (14), working with monkeys, dogs, and a mouse, reported that *E. coli* L-asparaginase was steatogenic to monkeys but apparently was not hepatotoxic to dogs or the mouse. They concluded that fatty infiltration of the hepatocytes seen in monkeys following i.v. administration of L-asparaginase does not appear to be dependent on either enzyme dose or specific activity and may reflect in part the initial nutritional state of the animal prior to treatment. It is difficult to make a direct comparison between their histologic studies on monkeys, dogs, and a single mouse and the findings of this investigation which were derived from different species (man and mouse) and different analytic methods (TLC and densitometry). Evidence obtained in this study would, in fact, suggest that in the case of mice given i.p. injections of L-asparaginase, there is an inverse relationship between the purity of enzyme and the resultant fatty infiltration of the liver.

TABLE III. Effect of High Specific Activity L-Asparaginase.

	% Crude lipids (dry wt.)	TG[a]
Control (physiological saline)	13.1	11.6
Group I (200 IU/kg × 5)	14.0	15.2
Group II (1000 IU/kg × 5)	13.7	14.6

[a] Percentage of crude liver lipids.

TABLE IV. Effect of High Specific Activity L-Asparaginase.

	% Crude lipids (dry wt.)	TG[a]
Control (physiological saline)	12.5	12.6
Group I (200 IU/kg × 5)	12.1	12.0
Group II (1000 IU/kg × 5)	11.9	12.0

[a] Percentage of crude liver lipids.

During the course of this investigation, enzyme of increasing purity, ranging in specific activity from 55 to 300 iu/mg of protein, was made available. In the early phases (Tables I and II) of this investigation a significant lipidosis (1.5- to 2-fold) was produced by the partially-purified L-asparaginase then available. This is evident in the increased percentage of total crude lipids as well as an increase in the percentage of triglycerides. However, as enzyme of increasing purity (250–300 iu/mg of protein) became available, varying dosage of L-asparaginase did not prove steatogenic to mice. This same effect was not observed in the analysis of liver tissues of L-asparaginase-treated patients. For while one patient had marked hepatic lipidosis after he received enzyme of only low

TABLE V. Hepatic Lipids of Leukemia Patients Treated with L-Asparaginase.

Patient	Purity of enzyme (IU/mg protein)	% Crude lipids (dry wt.)	TG[a]
F.H.	25–70	58.3	80.0
D.B.	70–140	24.5	56.0
J.C.	200–250	45.2	70.0
R.W.	200–250	46.4	72.0
N.T.	200–250	43.9	77.0

[a] Percentage of crude liver lipids.

purity (25–70 iu/mg of protein), the degree of fatty infiltration in several other patients, receiving enzyme of much higher purity (200–300 iu/mg of protein), was not significantly different. This might suggest that a difference in species susceptibility may account for the apparent steatogenic potency of high purity L-asparaginase seen in man and other primates, but not in mice or dogs. However, it should be recalled that in neither investigation was pure L-asparaginase employed; and therefore, the apparent hepatotoxicity may be due to some residual contaminant rather than the enzyme itself. Future investigations must utilize a still more highly purified L-asparaginase, must include analysis of liver lipids of leukemic patients not treated with this enzyme, and analysis of liver lipids of nonleukemic patients who have been treated with L-asparaginase in order to resolve this uncertainty.

Summary. Fatty infiltration of the liver following i.p. administration of partially-purified *E. coli* L-asparaginase was investigated using mice as experimental animals. Normal male C3H mice given varying dosage of low specific activity L-asparaginase over a 5-day period developed a marked hepatic lipidosis. Quantitative analyses and cytological studies confirmed this abnormal fat accumulation, in the form of triglycerides, compared with control animals given physiologic saline. By contrast, enzyme of high specific activity was not steatogenic to mice at the highest dose levels administered. Therefore, pure L-asparaginase per se does not appear to be steatogenic to mice. Quantitative analyses of liver tissues of a small group of L-asparaginase-treated leukemic patients showed marked hepatic lipidosis, regardless of amount or specific activity of enzyme administered. In fact, the severity of lipidosis seemed quite unrelated to the dose or purity of enzyme used. The limitations and possible implications of these findings are briefly discussed.

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