Hepatic Damage in Rats Fed Human Foodstuffs.* (22116)

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It is well established that laboratory animals fed one or another deficient diet may develop hepatic lesions resembling Laennec's cirrhosis. These observations have supported the view that an inadequate dietary intake contributes to the development of cirrhosis in human subjects. By and large, however, the experimental diets have had little resemblance to the diets eaten by such patients. The hypothesis that dietary deficiency is important in the pathogenesis of human cirrhosis would be strengthened if similar lesions appeared in animals fed foodstuffs resembling those eaten by patients with this disease. Gillman, Gillman, Mandelstam and Gilbert(1) were able to produce severe hepatic damage in rats fed corn meal and sour milk, a diet similar to that eaten by South African natives who commonly develop disease of the liver. The changes observed in rats included diffuse fatty liver, cirrhosis, and atrophy or hypertrophy of individual hepatic lobes without parenchymal necrosis. It seemed of interest to determine whether experimental animals would develop hepatic changes if fed diets similar to those of patients with cirrhosis of the liver in this country. The experiments to be described suggest that rats fed such diets may develop lesions which bear a similarity to those of human cirrhosis.

Method. Young male albino rats of the Wistar strain obtained from Carworth Farms, New City, N. Y., were housed in individual cages with open meshed bottoms. All rats were fed Purina dog chow and tap water *ad libitum* until the onset of the experimental period, at which time their mean weight was about 110 g. The rats were then fed one of 4 diets *ad libitum* as follows: Diet A (12 rats)—Purina dog chow and tap water. Diet B (45 rats)—spaghetti, olive oil and wine; the wine was diluted with an equal volume of water, Diet C (12 rats)—spaghetti, olive oil and 10% glucose in water, Diet D (12 rats)—spaghetti, olive oil and diluted wine with vitamin supplement. The composition of these diets is given in detail below. In 10 rats fed diet B and in all those fed diets C and D the amount ingested was measured grossly, taking into account spillage. For mechanical reasons the measurement of spillage was only approximate. As a result only a crude estimate of the actual dietary intake was possible. The animals did not have access to their feces or urine. Purina dog chow is composed of meat meal, dried skimmed milk, wheat germ, dried beet pulp, corn grits, cereal feed from corn and wheat, dried fig, soy bean oil meal, molasses, riboflavin supplement, brewer's dried yeast, Vit. A and D feeding oils, one per cent steamed bone meal, and one per cent iodized salt. According to the manufacturer, an average sample contains 23.0% protein, 6.0% fat, 9.6% ash, and 6.0% crude fiber. In addition, an average sample contains carotene, 9 mg, thiamin, 5.6 mg, and riboflavin, 5.0 mg per 100 g. Procino-Rossi brand elbow spaghetti is made of "high grade A-No. 1 semolina." Analysis by the Wisconsin Alumni Research Foundation demonstrated that 100 g of this spaghetti contained 10.9% moisture, 0.65% ash, 0.17% ether-extractable material, 13.38% protein, 0.24% crude fiber and 74.66% carbohydrate. There were 2.25 mg of cystine, 1.0 mg of methionine and 1.84 mg of choline per gram of spaghetti. This spaghetti is not fortified with vitamins. Analysis of the Parma California Burgundy was kindly provided by Dr. Aram Ohanesian, chief chemist of Cella Vineyards. One hundred ml contained 13.2% alcohol (by volume), 0.74 g of acid (as tar-

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TABLE I. Influence of Diet on Development of Hepatic Lesions in Rats.

Diet	Total animals	Rats sacrificed when free of symptoms	Rats which died or sacrificed when moribund	Hepatic lesions
A. Purina dog chow & water	12	12 at 158 to 343 days	0	Normal liver
B. Spaghetti, olive oil & wine	45	6 at 45 to 223 days	39 at 13 to 223 days	Necrosis, fatty infiltration and fibrosis
C. Spaghetti, olive oil & 10% glucose in water	12	4 at 139 to 343 days	8 at 191 to 273 days	Fatty infiltration and fibrosis
D. Spaghetti, olive oil, wine & vitamins	12	10 at 139 or 343 days	2 at 271 and 343 days	Normal liver

taric acid), 0.14 g of reducing sugar, 0.19 g of tannins and 0.025 g of protein. The pH of the undiluted wine was 3.65. The wine was diluted with an equal volume of water and was fed in water bottles. No analysis was available of the olive oil used, but pure olive oil is said to be composed entirely of lipids (2) and is devoid of choline(3). Olive oil is notably rich in unsaturated fats; the oil used at no time appeared to be rancid. The vitamin supplement in Diet D consisted of 1 ml of an aqueous suspension containing 125 μg of thiamine. 100 μg of riboflavin, 125 μg of pyridoxine and 750 μg of calcium pantothenate per ml added to each 100 ml of diluted wine. In addition, one drop of cod liver oil USP was added to the spaghetti once weekly.

Results. The effect of the various diets tested is summarized in Table I. Diet A (Purina dog chow and tap water). These animals grow rapidly and remained healthy in appearance and behavior. All were sacrificed while in good condition from 158 to 343 days after the onset of the experimental period. Autopsy showed no significant abnormality. Histologically the liver and kidney were normal in every instance.

Diet B (spaghetti, olive oil, and wine). The rats gained weight at a progressively diminishing rate for about 30 days and then gradually lost weight until they died or were sacrificed. Six rats were sacrificed without obvious symptoms from 45 to 223 days after the start of the experiment, while 39 died or were sacrificed when moribund at periods ranging from 18 to 223 days.

Seven of the 45 animals showed severe diffuse hepatic necrosis. This lesion was noted in 4 of the 12 rats which died within 115 days of the start of the experiment and in only 3 of the 33 rats which survived longer than this. The necrosis involved all lobules and affected small groups of cells. The latter were usually shrunken and presented acidophilic homogenous cytoplasm with indistinct borders, while the nuclei were reduced in size, pyknotic, and often fragmented. There was also focal coagulation necrosis with no nuclear content. The necrotic cell groups alternated with fairly well preserved hepatic cells, whose cytoplasm was vacuolated. Marked fatty degeneration was present in all the necrotic livers. No leukocytic reaction was evident. The liver cords were commonly fragmented and the sinusoids prominent. In a few animals there were foci of necrosis in which there was complete loss of liver architecture. These lesions were associated either with hemorrhage or hyperemia. There were no distinct nodular regenerative foci. There was slight to moderate fibrosis, bile duct proliferation, and lymphocytic exudate in the portal areas. However the fibrous reaction within the lobules was mainly of condensation type. In 8 additional rats on this diet there were slight to moderate grades of fatty degeneration or fatty infiltration, mainly periportal in location and in a few instances associated with a mild degree of fibrosis.

Diet C (spaghetti, olive oil, and 10% glucose in water). These rats gained a small amount of weight for a month or 2, and then their weight either remained relatively stationary or decreased slightly until they died or were sacrificed. Four animals in this group

were sacrificed without symptoms at 139 to 143 days and 8 died or were sacrificed in a moribund state at 139 to 273 days. In the rats in this group, parenchymal necrosis was not observed. However there were 5 animals with moderate or marked fatty infiltration of the liver, principally periportal, with formation of the signet-ring-type of hepatic cell. All were associated with a mild degree of periportal fibrosis.

Diet D (spaghetti, olive oil, wine, and vitamins). These rats either gained weight slowly but steadily or reached a maximum from which their weight did not decline, unlike the rats fed Diets B and C. Ten rats were sacrificed without symptoms at 139 to 343 days, one died at 271 days, and one was sacrificed when moribund at 343 days. There were no significant lesions of the liver in this group. No hepatic cell necrosis was found. There was one animal with a slight degree of periportal fat infiltration.

During the first 60-day period, at a time when none of the rats on measured diets had died or been sacrificed, a group of 10 rats fed diet B ate an average of 9.6 g of spaghetti and drank 14.1 ml of diluted wine per day; spillage prevented an accurate measure of the olive oil ingested. Rats offered Diet C averaged 7.9 g of spaghetti and 19.4 ml of glucose daily, and rats fed Diet D, 9.4 g of spaghetti and 10.8 ml of wine, as well as vitamin supplements. Thus rats eating a diet of spaghetti, olive oil and wine, with or without vitamin supplements, consumed 17 mg of choline, 18 mg of methionine, and 21 mg of cystine daily. Those fed a diet in which glucose was substituted for wine consumed 14 mg of choline, 15 mg of methionine, and 18 mg of cystine daily. It cannot be assumed, however, that the rats continued to ingest these quantities of food at a later time.

Discussion. Chronic alcoholism is common in patients with Laennec's cirrhosis of the liver in the United States. Nonetheless, it has not been possible to demonstrate that the administration of alcohol to experimental animals leads to hepatic lesions. Within recent years, attention has been drawn to the possible part played by chronic dietary deficiency in the pathogenesis of cirrhosis in chronic alcoholic patients. This view has been supported by the apparent beneficial effects of a nutritious diet in patients with this disease and by the production of hepatic lesions in experimental animals. In general, the lesions in experimental animals have been of two types, namely, fatty infiltration and fibrosis, prevented by choline or methionine, and necrosis of liver cells, prevented by cystine or methionine(4-6). However, the diets used in most experimental studies have been composed of foods not ordinarily eaten by human beings.

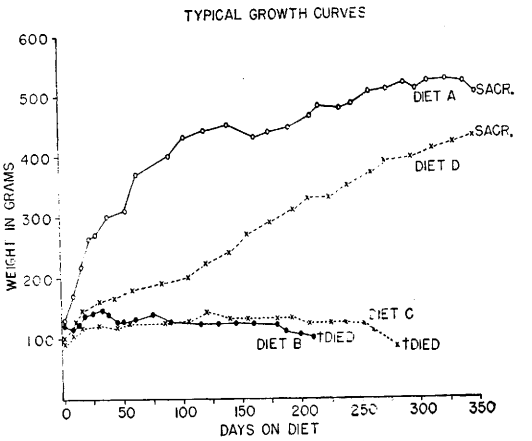

FIG. 1. Typical growth curves of rats fed Diets A, B, C and D.

In the present experiments, rats were fed diets containing foods commonly used by man. These diets consisted of some of the foodstuffs consumed by patients with cirrhosis who are of Italian extraction, an ethnic group in which this disease occurs more commonly than in the rest of the population of the United States(7). In these experiments, both diffuse necrosis of liver cells and fatty infiltration and fibrosis were noted; the relative frequency of the lesion depending upon the nature of the diet and the duration of survival. Rats fed spaghetti, olive oil and wine (Diet B) developed necrosis of liver cells, fatty degeneration and fibrosis. Necrosis was relatively more frequent in those rats which died within the first 4 months of the experiment than in those which survived longer than this time. Rats fed spaghetti, olive oil and wine, supplemented by a vita-

min mixture not containing tocopherol (Diet D) had no significant lesions even though they did not appear to ingest more spaghetti than the rats fed the same diet without vitamins. The substitution of glucose for wine, without the addition of vitamins (Diet C) somewhat prolonged the time of survival, and at the same time modified the nature of the lesion so that fatty infiltration and fibrosis developed rather than necrosis. It is noteworthy that the glucose-fed rats obtained relatively more of their calories from glucose and less from spaghetti, when compared to wine-fed rats. The spaghetti was the sole source of choline and methionine.

Summary. Significant hepatic lesions, consisting of diffuse parenchymal necrosis and marked fatty infiltration, have been produced in rats fed diets of spaghetti, olive oil and wine or glucose solution. Both lesions were associated with a moderate degree of fibrosis. The specific dietary factors responsible for the changes were not ascertained.

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Lipides Distribution and Phospholipide Turnover in Tissues of Rabbit Shortly After Growth Hormone. (22117)

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The concept that arteriosclerosis is a disease of metabolism has focused attention on endocrine control of lipide metabolism and their relationship to development of arterial degenerative lesions. It is increasingly evident that for elucidation of this relationship more information is required of the modes of action and nature of effects of hormones on lipide metabolism in different tissues. Certain adrenocortical steroids(1-3) and epinephrine (3-5) were reported to exert an influence on lipide distribution in tissues and development of aortic degenerative changes. There is ample evidence that growth hormone causes accelerated mobilization and catabolism of endogenous fat(6-10), but there is little information concerning the effect of growth hormone on lipide partition in tissues. Recently, Greenbaum and MacLean(7) studied distribution of lipides in plasma and liver of intact rats at intervals after treatment with growth hormone. Greatest changes occurred 6 hours after growth hormone when plasma and liver neutral fat and plasma phospholipide were significantly increased. The authors suggested that increased plasma phospholipide could be explained by accelerated synthesis of this lipide since Geschwind *et al.*(11) reported increased turnover of liver phospholipide in normal and hypophysectomized rats after 14 days or more of treatment with growth hormone. Mayer and Jones(12) reported a significant, but slight, increase in blood cholesterol of mice after 2 weeks treatment with growth hormone.

The present study was undertaken to determine concurrently lipide partition and phospholipide turnover in plasma, liver and aorta of intact rabbits a few hours after treatment with growth hormone. Knowledge of phospholipide-cholesterol-neutral fat relationships and phospholipide metabolism in the above tissues was a necessary preliminary to a study of the influence of growth hormone on lipide