ner, an antigen-antibody complex is formed which circulates in the parabiotic pair and becomes a new antigen to which antibodies are formed. This secondary antigen-antibody complex could then cause the disease in the secondary partner similarly to the glomerulitis caused by injected beef gamma globulin(10).

It is possible that a similar mechanism plays a role in causation of subacute or chronic glomerulonephritis. Hume and Merrill(11) have reported several instances in which acute glomerulonephritis occurred in a healthy kidney transplanted from an identical twin to a patient with far advanced glomerulonephritis. Much more study will be needed to evaluate the significance of the secondary antibody in the genesis of chronic glomerulonephritis.

Summary. The glomerular basement membranes in kidneys of rats made nephritic by antirat-kidney rabbit serum show immediately the presence of rabbit gamma globulin when examined with a fluorescein labelled antirabbit gamma globulin serum. Rat gamma globulin, presumably rat antibody, appears only after 6 days on these membranes. The parabiosis of such nephritic rats to healthy rats leads to a mild but clear cut nephritis in the healthy partner with proteinuria and typical histologic changes. Rat gamma globulin, presumably antibody, but not rabbit gamma globulin can be shown on the glomeruli of such secondarily diseased animals. The parabiosis of 2 normal rats does not lead to such a deposition of antibody or to clinical disease. Thus the primary nephritic partner seems to form an auto-antibody against its own altered kidney tissue. This antibody is then in turn able to attack the healthy kidney tissue of the secondary partner.

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Received July 8, 1960. P.S.E.B.M., 1961, v106.

Hepatic Alterations in Rats Fed 1,2-dihydro-2,2,4-trimethyl-quinoline, *Flectol H.* (26222)

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A variety of agents is capable of causing hepatic injury with attendant fatty metamorphosis, loss of cytoplasmic basophilia, and hepatic necrosis. Severe acute or chronic injury may result in fibrosis, cirrhosis, parenchymal and biliary-duct hyperplasias, and tumor formation. Relationships among these phenomena are complex and variable. Thus, signs of injury may be reversible or progressive, while tumors may or may not be preceded by fibrosis and cirrhosis. Exploration of apparently unrelated pathways by which diverse agents produce similar ends may disclose common basic mechanisms. This report calls attention to another means for pro-

TABLE I. Part A. Liver Weight-Body Weight Ratio.

Day	Weanlings (range)	Adults (range)
1	.52-.54	.38-.45
2	.58-.65	.43-.46
3	.59-.74	.45-.52
4	.61-.73	.45-.48
5	.81-.85	.53-.60
6	.81-.98	.55-.70

ducing many of the above-mentioned hepatic changes.

During toxicological studies on a polymer of 1,2, - dihydro - 2,2,4, - trimethyl - quinoline, *Flectol H,* we noticed histologic changes limited to the livers of test rats. These included marked fatty change frequently limited to, or most prominent in, the midzonal area of the hepatic lobule, cellular alterations, pigmentation, and biliary-duct hyperplasia.

Materials and methods. Flectol H, a polymer of 1,2, - dihydro - 2,2,4 - trimethyl - quinoline, is an industrial antioxidant listed in the 29th edition of Monsanto's Catalogue of *Chemicals, Plastics, and Petroleum Products.* It is supplied as a powder and used in experimental diets by mixing with Fox Chow at levels of 0.01%, 0.1%, 1.5%, and 3.0%, by weight. White rats of the Rochester Wistar strain were used.

Part A. Eighteen male weanling rats of 60 to 75 g and 18 adult male rats of 150 to 175 g were given Fox Chow with 1.5% *Flectol H* by weight. After 24 hours, daily sacrifices of 3 rats from each group were performed for the next 6 days.

Part B. Twenty male and 20 female rats of 50 to 60 g were placed on control diet and on diets containing 1.5% and 3.0% *Flectol H.* Five rats from each group were sacrificed at weekly intervals for one month.

Part C. Thirty-five male and 35 female rats were maintained for 2 years on the following diets: control, 0.01%, 0.1% and 1.5% *Flectol H.*

In part C, rats were weighed weekly while the others were weighed at time of sacrifice. Animals were allowed *ad libitum* access to diet and water. Rats were sacrificed by decapitation, autopsies performed, and organ weights recorded. Only 3 males and 5 females given the 1.5% diet in the 2-year study survived to sacrifice. This represented decreased survival as compared to the other groups. Tissues fixed in 20% neutral formalin were stained with hematoxylin and eosin. Selected sections were examined with polarized light and with fluorescent light microscopy. Fat stains were prepared on fixed frozen sections of liver using oil red O. PAS, iron stains, acid-fast stains, and fat stains on paraffinized sections were performed on selected sections.

Results. Gross observations. Parts A and B. Enlarged, pale, soft livers were evident within 2 or 3 days and markedly obvious after one week. The increase in hepatic weight was due in part, at least, to increased hepatic fat (Tables I and II). In weanling rats the total hepatic lipid increased 60% in 5 days while it stayed nearly constant in adult rats.* Slight growth lag was seen in the 1.5% diet group; the 3.0% rats failed to gain weight and did not survive beyond 3 weeks. *Part C.* Of the rats at the end of the 2-year study, those of the (male and female) 1.5% groups showed enlarged livers; female rats of the 0.1% diet group may also have had some liver enlargement. Three of the 5 female rats in the 1.5% diet group had one to several gross nodules in their livers. Nodules were 3 to 10 mm in diameter, yellow-gray or gray-white, granular, moderately firm, and occasionally umbilicated. Rarely, a red-brown nodule of several millimeters in

TABLE II. Part B. Liver Weight-Body Weight Ratio (Ranges).

	Control		1.5% diet group		3% diet group	
Week	♂	♀	♂	♀	♂	♀
1	.48-.60	.46-.53	.75- .81	.68-.74	.95-1.15	.79- .93
2	.48-.50	.47-.52	.74-1.01	.55-.95	.95-1.30	.89-1.32
3	.38-.63	.47-.55	.72- .86	.68-.81	1.34-1.44	1.06-1.41
4	.43-.51	.46-.48	.61- .73	.62-.77	—	—

* Determinations performed by Dr. Frances Haven.

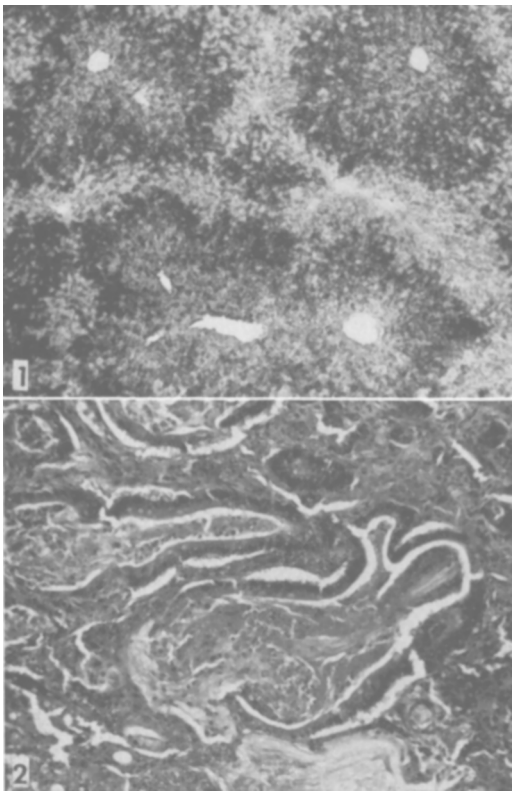

FIG. 1. Liver showing predominant midzonal distribution of fat (oil red O × 48).

FIG. 2. Atypical bile-duct hyperplasia resulting from chronic administration of *Flectol H* (H & E × 120).

diameter was noted. Where gross nodules were not present, whitish areas of less than one millimeter in diameter were seen in the livers of the rats on a 1.5% *Flectol H* diet. Vascular channels were frequently dilated. No cirrhosis was seen. One rat of the 0.1% diet group had numerous yellow-gray, firm, 2 to 3 mm nodules of metastatic tumor in the mesentery arising from the pancreas.

Microscopic observations. Parts A and B. A minute amount of fat was seen in Kupffer cells at the end of 24 hours. From the second to sixth days weanling rats showed increasing amounts of hepatic fat in midzonal and occasionally in centrolobular or periportal areas while adults showed inconstant lesser amounts of fat (Fig. 1). During the period from one week to one month the amount of fat increased as its distribution became more diffuse. Rats of the 1.5% diet group tended to have more obvious midzonal fat while those of the 3% diet group showed severe diffuse fatty change. Cellular changes included loss of cytoplasmic basophilia, hyaline degeneration, nuclear enlargement, and increased numbers of double nuclei, all more obvious in the 3% diet group of rats. Biliary-duct hyperplasia was seen in the 3% diet group at the end of 3 weeks and at the end of 4 weeks in the 1.5% diet group. Often it was difficult to distinguish the hepatic or biliary origin of clusters of proliferating cells. One rat only exhibited bile plugs within canaliculi.

Part C. Most marked change was found in the 1.5% diet group of rats. Gross nodules corresponded to the most atypical biliary-duct hyperplasia. These consisted of irregular acini lined by columnar cells with basal nuclei and often showing goblets of mucoid material in the cytoplasm. Lumens contained mucin, eosinophilic debris, and leukocytes. Frequently, stratification of nuclei and mitoses were seen in the most atypical acini. Within the large amount of surrounding fibrous connective tissue were small regenerative islands of liver cells and numerous inflammatory cells (Fig. 2). The rest of the liver revealed smaller foci of duct hyperplasia, variation in hepatic cell size, and enlarged hepatic cells with eosinophilic cytoplasm. Globular yellow-brown pigment was prominent in both Kupffer cells and parenchymal cells. Special stains suggest that this pigment is a lipofucsin.

Discussion. Localization of fatty metamorphosis and toxic hepatic injury within the hepatic lobule may be limited to the central or periportal zones in response to a variety of agents(1,2). Rarely, as in the responses to beryllium injection(3) or to chloroform by hyperthyroid rabbits(4), fatty change and necrosis are limited to the midzone of the lobule. *Flectol H* is capable of producing midzonal localization of fat without significant hepatic parenchymal necrosis. No single hypothesis based on either circulatory or on functional differences within the lobule would seem to explain the simultaneous occurrence of central, midzonal, and periportal fatty change in response to *Flectol H*.

The biliary-duct hyperplasia and hepatic cell alterations are similar to the effects of butter yellow(5,6,7) as well as of a number of other agents(8,9). This atypical proliferation of biliary ducts and of fibrous tissue is called cholangiofibrosis and is considered to be a precursor of biliary-duct carcinoma(7). If analogy to butter yellow is extended, it is probable that dietary alterations will cause more rapid onset of these lesions.

Such reactions to feeding an antioxidant raise questions as to mechanism and site of action. Relations worthy of investigation are those involving Vit. A, Vit. E, and choline and methionine. Further work may also be of value in elucidating lobular localization of hepatic injury and the responses of biliary-duct epithelium and hepatic cells.

Summary. *Flectol H,* a polymer of 1,2-dihydro-2,2,4-trimethylquinoline, when fed to rats produced fatty change frequently localized to the midzonal area of the hepatic lobule. Chronic administration led to alteration of hepatic cells, necrosis, and atypical biliary-duct hyperplasia.

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Received August 30, 1960. P.S.E.B.M., 1961, v106.

Extratrabecular Crystallization in Rickets; Effects of Vitamin D, Calcium and Norethandrolone.*† (26223)

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Recent observations have revealed the presence of abnormal mineral deposits adjacent to the bone trabeculae of chicks fed a diet containing 50% ground seeds of *Lathyrus odoratus*(1). Another series of experiments, yet unpublished, involving suckling rats injected with the toxic agent of the lathyrus seed, Beta-amino-propionitrile, has produced similar extratrabecullar masses. In both instances and also in a recently observed specimen of human osteogenesis imperfecta, the large round crystals were best seen in dark-phase microscopy; they were birefringent in polarized light. The bone trabeculae were immature, fibrillar; they were deficient in polysaccharides and minerals. A current series of experiments involving undemineralized sections of tibiae from rachitic chicks variously treated has revealed a similar condition.

Materials and technics. Five groups of 10 newborn chicks/group were fed the low Ca AOAC diet(2) without Vit. D. After one week the diet was supplemented in 4 groups as follows: (a) $Ca_3(PO_4)_2$ 1%; (b) Vit. D^3 1000 units %; (c) Norethandrolone (Nilevar, Searle) 0.5 g/kilo; (d) Norethandrolone as in (c) with $Ca_3(PO_4)_2$ as in (a). Group 5 was unmodified. At 2 weeks of age, the untreated birds were suffering from severe rickets as judged by tarso-metatarsal radiographs(3), bone ash, Ca^{45} intestinal absorption(4) and by the histological picture of cartilage and bone. Calcium and norethan-

* Contribution No. 46, from Animal Research Inst., Research Branch, Canada Dept. of Agriculture, Ottawa.

† The authors are indebted to Mrs. L. F. Bélanger for skilled technic and to the Canada Dept. of Agriculture for financial assistance.