in food quality in subsequent years[15]. This in turn causes heavy larval mortality in early instars of the dark colormorph which is most vulnerable to nutritional stress[13]. During the subsequent decline in population density the proportion of the more robust intermediate color type increases until it predominates at lowest population densities, some 4 years after defoliation[14]. After relaxation of the selection pressure against the dark fitness type, coinciding with the recovery of the larch tree, assortative mating by the small proportion of the true-breeding dark food specialists remaining at minimum density of the cycle might represent the important driving mechanism which contributes to the rapid increase in its frequency in the population, and subsequent proliferation[14]. Pheromone polymorphism could thus be considered an important fitness characteristic of the larch budmoth permitting it to exploit continuously an inconsistent food resource.

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Chronic active hepatitis in mice induced by 3-hydroxy-4-pyrone[1]

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Summary. Chronic active hepatitis was selectively induced in mice by the feeding of a diet containing 3-hydroxy-4-pyrone (0.5% by weight) for periods of 6 weeks and longer. This model should be of particular value in elucidating the pathogenesis of drug-induced forms of chronic active hepatitis. Maltol (3-hydroxy-2-methyl-4-pyrone) did not produce any liver lesion.

Chronic active hepatitis (CAH), an inflammation of the liver continuing without improvement for at least 6 months, is a pattern of progressive hepatocellular damage rather than a single disease and has several known causes in humans, including autoimmunity, chronic viral hepatitis (types B and non-A, non-B), alpha-1-antitrypsin deficiency, Wilson's disease, alcoholism and a few therapeutic drugs[3,4]. Whatever the cause, its diagnosis depends on the identification of the characteristic histological lesion consisting of periportal piecemeal necrosis and a dense infiltrate of lymphocytes and plasma cells and in severer cases on the presence of the additional features of portal-to-portal and portal-to-central bridging necrosis and fibrosis with isolation of groups of liver cells within the cellular infiltrates. The pathogenesis of drug-induced CAH is unknown and an experimental model is lacking. A liver lesion resembling CAH was observed in an earlier study of Swiss mice fed 3-hydroxy-4-pyrone, a known inhibitor of catechol methyl transferase[5] and of thyroid peroxidase and other peroxidases[6]. Here we report studies on the nature and progression of the lesion induced in mice by chronic feeding of 3-hydroxy-4-pyrone and on the effects of subsequent withdrawal of the substance from the feed. Maltol (3-hydroxy-2-methyl-4-pyrone), a commonly used flavoring agent in foods, was fed to mice in a parallel study but did not produce any liver lesions.

Materials and methods. Female Swiss mice, 20–25 g, were placed in separate cages in groups of eight. Food and water were available ad libitum. Mice were weighed weekly. 3-Hydroxy-4-pyrone was prepared as previously described[6]. Maltol was a gift from the Chemical Division, Pfizer, N.Y. Each compound (0.5% wt/wt) was added to ground mouse pellets as described in detail elsewhere[7]. The mouse pellets used (Clark King GR2 pellets, Victorian Wheatgrowers Corporation, Melbourne) had the following composition (manufacturer's analysis): protein 20%, fat 3.0%, fiber 3.0%, calcium 0.7%, phosphorus 0.6%, iodine 0.6 ppm, manganese 115 ppm, zinc 90 ppm, iron 100 ppm, copper 10 ppm.

Mice were killed by exsanguination under ether anesthesia at the times shown below. Livers were weighed and separate portions of the left and median lobes were fixed in phosphate-buffered 10% formalin and processed to paraffin sections which were stained with H&E and by the Gordon and Sweets method for reticulin. Other tissues processed similarly included the brain, skeletal muscle, heart, lungs, gut, kidneys, thymus, lymph nodes, spleen and bone marrow. The criteria used for the diagnosis of CAH were strictly histological and no time factor comparable to that required in human cases was taken into consideration[3,4].

The number of mice used was determined by the limited supply of the test compounds. Each test diet was fed to 24 mice. An equal number were fed normal mouse pellets. In the case of 3-hydroxy-4-pyrone, 4 test and 2 control mice were killed at 3, 6, 9 and 12 weeks and 2 test and 2 control mice were killed at 16 and 21 weeks. At 21 weeks the 4 remaining mice were returned to normal pellets to study the effect of withdrawal of the test compound. Two of these mice were killed at 10 and 20 weeks after removal of the test compound from the diet, i.e. 31 and 41 weeks after the start of the original experiment.

Results. The control and test groups of mice gained weight progressively and looked healthy. None died. The liver to body weight ratio remained constant. The livers of control mice

showed no histological abnormalities. Except for the changes in the livers of the test mice, all other tissues examined were histologically normal.

The livers of all 4 test animals killed at 3 weeks showed confluent centrilobular liver-cell fallout with occasional portal-to-central bridging associated with a moderate lymphohistiocytic response within the lobules. Portal tracts contained a slight lymphocytic infiltrate. Liver cells showed slight nuclear enlargement and a few were in mitosis. Of the 4 mice killed at 6 weeks, 3 showed progression of the changes noted at 3 weeks to those of CAH and included piecemeal necrosis as well as central-to-portal and portal-to-portal bridging necrosis as well as piecemeal necrosis. Moderately dense portal, periportal and panlobular cellular infiltrates of lymphocytes, histiocytes, plasma cells and polymorphs were present. The liver of the fourth mouse showed only mild lobular and moderate portal inflammation.

3 of 4 mice killed at both the 9th and 12th week showed the presence of some multilobular scars and portal-to-portal and central-to-portal septa surrounding incompletely formed parenchymal nodules (fig. 1). There was extensive periseptal and periportal piecemeal necrosis with trapping of liver cells within dense mixed cellular infiltrates composed of lymphocytes, plasma cells, ceroid-filled macrophages and a few polymorphs (fig. 2). Trapped liver cells and hepatocytes in the regenerative nodules were enlarged and had big nuclei (fig. 3). Elsewhere the liver showed small regenerating hepatocytes and mitotic figures, especially in central and midlobular zones and no inflammation. The remaining mouse killed at each 9 and 12 weeks showed only mild patchy piecemeal necrosis without distortion of lobular architecture. 2 mice killed t each 16 and 21 weeks showed severe lesions similar to those seen at the 12th week. The 2 mice killed at each 10 and 20 weeks after reversal of the dietary regimen showed considerable regression of the lesions with residual portal-to-portal fine fibrous bridges and portal lymphoplasmacytic aggregates and collections of ceroid-filled macrophages. These changes could be categorized as chronic persistent hepatitis with residual scarring (fig. 4).

Discussion. Among the drugs which have been reported to be associated with CAH in man are: oxyphenisatin, alphamethyldopa, nitrofurantoin, dantrolene, isoniazid and paracetamol[3]. Only a very minor percentage of patients taking these drugs develop the liver lesion and in the great majority the disease regresses on withdrawal of the drug. When fed to mice, 3-hydroxy-4-pyrone selectively induces CAH with bridging hepatic necrosis within 6 weeks and withdrawal of the chemical from the diet after 12 weeks of feeding leads to regression of the lesion with residual scarring. To our knowledge, no previous experimental model of drug-induced CAH has been described.

The pathogenesis of drug-associated CAH in man has not been defined. Some drugs may cause liver cell necrosis either directly or through an active metabolite, which when it is prolonged could lead to chronic toxic liver injury. Other drugs may behave as immunostimulants leading to altered immunoreactivity, initiating a process of continuing hepatocellular necrosis. It has been proposed that certain drugs may serve as 'trigger factors' in susceptible individuals, provoking an autoimmune reaction that produces tissue damage in the liver[8]. Of all the experimental animals, the mouse is one in which the

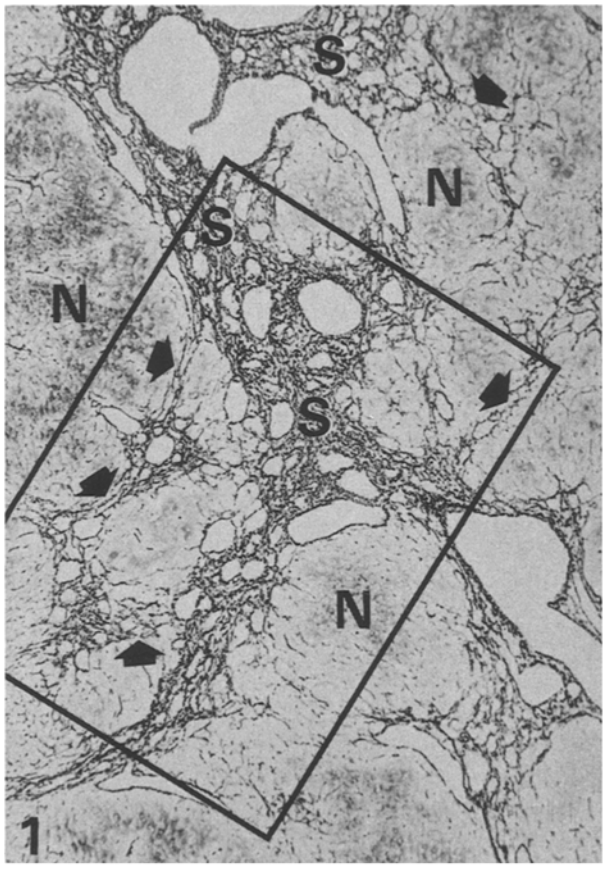

Figure 1. Liver of mouse fed the diet containing 3-hydroxy-4-pyrone for 12 weeks shows multilobular scars (S). Fibrous septa (arrows) following bridging necrosis incompletely surround regenerative nodules (N). Reticulin, ×45.

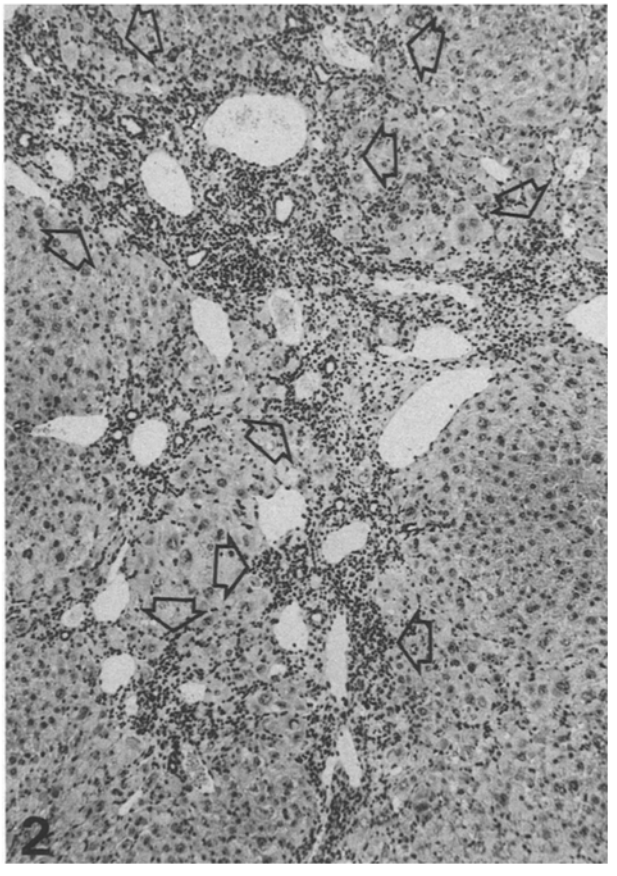

Figure 2. Closer view of the area marked out in figure 1 shows crowding of the vascular structures related to multiple lobules which have undergone collapse and fibrosis following necrosis. Dense inflammatory cell infiltration is seen in portal tracts and in periportal and periseptal locations (arrows). H&E, ×73.

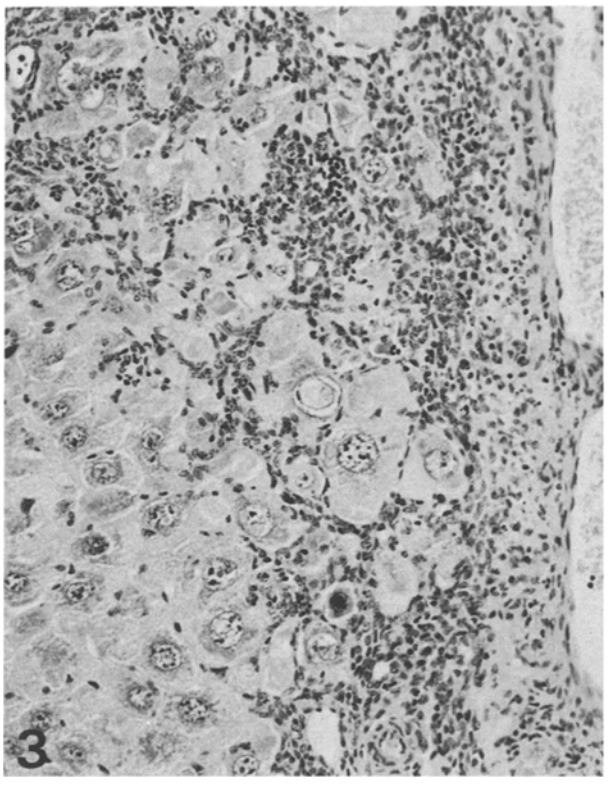

Figure 3. Higher power view showing extensive periportal piecemeal necrosis with trapping of surviving liver cells within the dense mononuclear infiltrate. Several liver cells show cellular and nuclear enlargement. H&E, ×156.

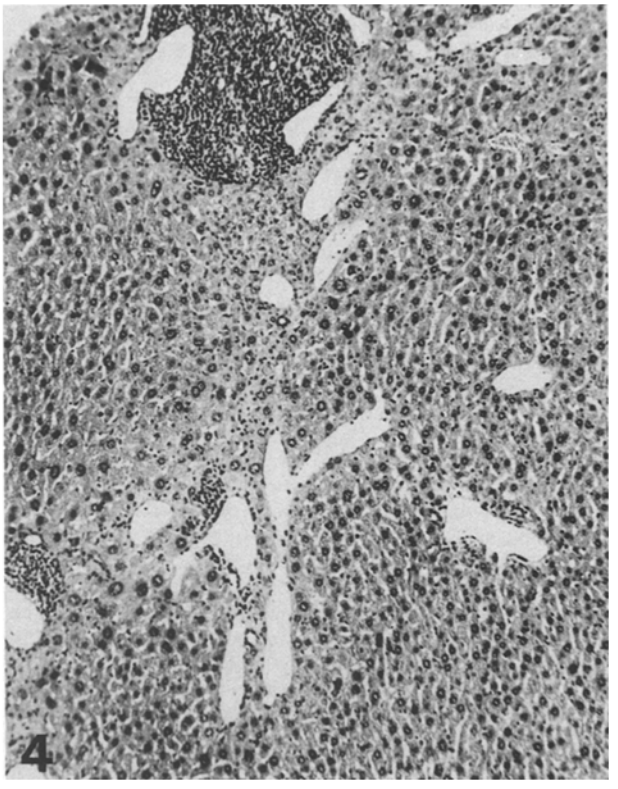

Figure 4. Liver of mouse fed 3-hydroxy-4-pyrone for 21 weeks followed by normal mouse pellets for the next 20 weeks shows regression of CAH with residual scarring, crowding of vascular structures and a persistent lymphoid infiltrate. Compare with figure 2. H&E, ×75.

immune system has been characterized in detail and is also the species in which several inbred, recombinant and congenic strains are available. Therefore the mouse model described here should provide a valuable tool for defining any immunological and/or genetic determinants of CAH.

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Metabolic implications in the elevation of serum activity of intestinal alkaline phosphatase in chronic renal failure

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Summary. The activity of intestinal isoenzyme of serum alkaline phosphatase was evaluated in 21 non-dialyzed patients with advanced renal failure and in 52 patients on regular hemodialysis. In patients without hepatopathy, a significant inverse correlation was found between the enzyme activity and serum calcium levels. Hepatopathy was the most significant variable influencing the enzyme activity in patients on dialysis. Secondary hyperparathyroidism and a decreased rate in enzyme elimination should be assessed for the above-normal activities of intestinal ALP in serum in chronic renal failure.

Elevation in the serum activity of intestinal isoenzyme of alkaline phosphatase (EC 3.1.3.1) has been described in patients on regular hemodialysis[1-3]. The present investigations were designed to determine the factors which influence the changes in the enzyme activity in chronic renal failure.

Patients and methods. Measurements were made in 21 non-dia-