the drug dose used was achieved by serial passage and treatment with a constant dose of MTX for only two generations. By comparison, dihydrofolate reductase activity in the FR-1 subline rose to about ten times the original level.[1]

It may be noted that fewer treatments were administered in each transfer generation with the ascitic sublines of L1210, in which more passages were required to achieve resistance to MTX.[3-5,7] Thus establishment of resistance of a cell line with a given mutation frequency to a given dose may be a function of the total duration of exposure. At the end of a course of treatment of systemic leukemia L1210 for at least 15 days, the tumor exhibits an increase in dihydrofolate reductase levels, which in turn may contribute to the failure of therapy. The results of treatment of murine leukemia with MTX thus parallel the therapeutic situation in man and suggest use of this technique for the study of drug resistance in the clinical situation.

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Persistent reduction of serum bilirubin levels after treatment of Gunn rats with some acidic compounds

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THE EXCRETION of bilirubin is largely dependent on its conjugation with glucuronic acid to form the water soluble glucuronide which is then excreted into the bile. Gunn rats exhibit a type of hereditary jaundice due to their inability to conjugate bilirubin with glucuronic acid. This jaundice is characterized by fairly constant serum levels of unconjugated bilirubin of 5–15 mg per cent which persist throughout the life-time of the animal. A single dose of a variety of acidic compounds (e.g. sulphisoxazole,[1] salicylate[2]) have been shown to reduce the level of serum bilirubin in Gunn rats presumably by competitive binding on albumin which results in displacement of bilirubin into other tissues,

including brain.[3] This effect persisted for as long as the acidic compound remained in the blood and a reciprocal relationship between plasma concentration of bilirubin and drug has been demonstrated by Johnson.[1] A marked reduction in serum bilirubin levels which persists for several months after the end of treatment with either I.C.I. 54,450 [2-(4-chlorophenyl)thiazo-4-yl acetic acid] or salicylate has now been demonstrated.

Three groups of three male and three female Gunn rats were treated as follows: Group I untreated controls; Group II received I.C.I. 54,450 (70 mg/kg body wt.) per os daily for 28 days; Group III received salicylate (200 mg/kg body wt.) per os daily for 26 days.

The serum levels of bilirubin (estimated using a Boehringer Test Combination 15944TBAA) were followed throughout the dosing and for a further 10 weeks after salicylate treatment and 17 weeks after I.C.I. 54,450 treatment had ended.

Both compounds caused an initial rapid fall in serum bilirubin levels from about 10 mg per cent to about 4 mg per cent. This reduction persisted throughout the period of dosing. However, when dosing stopped the serum bilirubin levels rose only slightly, to about 6 mg per cent and were maintained at that level until the experiment was terminated 10 or 17 weeks later (see Figs. 1 and 2).

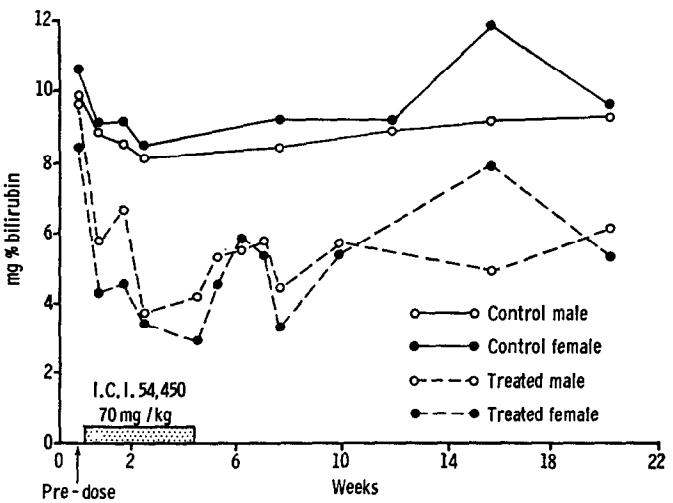

FIG. 1. Effect of I.C.I. 54,450 on serum bilirubin levels in Gunn rats.

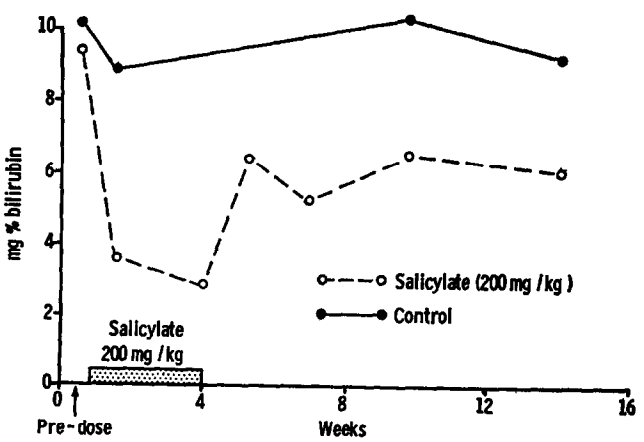

FIG. 2. Effect of salicylate on serum bilirubin levels in Gunn rats.

The failure of the bilirubin serum levels to return to the original level suggests that some permanent alteration in the Gunn rats' capacity for excretion of bilirubin has been induced by treatment with salicylate or I.C.I. 54,450. In Gunn rats bilirubin is broken down to polar diazo-negative derivatives which are excreted in the bile, or unconjugated bilirubin is transported directly across the intestinal mucosa into the gut and a little is excreted in the urine.[4] These routes, although presumably less efficient excretory mechanisms than conjugation with glucuronic acid, result in maintenance of a serum bilirubin concentration of 5–15 mg per cent in a steady state in the Gunn rat. It is unlikely that a direct effect on the albumin binding sites or on the rate of degradation of haem caused the prolonged reduction of serum bilirubin levels as the effect persists well beyond the turnover time for haem or albumin in the rat.

The reduction of serum bilirubin levels may therefore be due to irreversible stimulation of one of the pathways of excretion already available or the result of activation of bilirubin-UDP glucuronate glucuronyltransferase (EC 2.4.1.17) in the Gunn rat. Activation of UDPG transferase in the Gunn rat by diethylnitrosamine treatment both *in vitro* and *in vivo* has been demonstrated if *p*-aminophenol or paracetamol but not if *p*-nitrophenol, phenolphthalein or menthol are used as the aglycone.[5,6]

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