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Effect of *N*-hydroxy-2-acetylaminofluorene on ribonucleic acid and deoxyribonucleic acid synthesis

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ALTHOUGH the mechanism of liver carcinogenesis by 2-acetylaminofluorene (AAF) and its *N*-hydroxy metabolite (*N*-hydroxy-AAF) has not been fully elucidated, two general effects of the carcinogen have been recognized as being important in tumor induction. An early effect of the carcinogen is the acute hepatotoxicity (massive periportal necrosis) which is induced by *N*-hydroxy-AAF.[1] The hepatotoxicity, probably caused by the formation of AAF-*N*-sulfate in the liver, is followed by compensatory cell proliferation,[2] which is believed to be necessary for tumor formation.[3] Whether the role of hepatotoxicity is merely to induce cell proliferation, or is actually a part of the "initiation" step[3] is unclear. This problem has stimulated numerous investigators to study the initial effects of AAF and *N*-hydroxy-AAF on various biochemical processes. Because of their central role in the control of cell function, RNA and DNA synthesis have been given considerable attention. Presently there are conflicting reports in the literature on the effect of *N*-hydroxy-AAF on RNA synthesis in rat liver cells. Zieve and Gutmann[4] found that within 1 hr after a single injection of *N*-hydroxy-AAF to rats, RNA polymerase activity of isolated liver cell nuclei was inhibited as much as 80 per cent. Zieve[5] later reported approximately 70 per cent reduction in orotic acid incorporation into liver RNA *in vivo* 2 hr after an intraperitoneal injection of *N*-hydroxy-AAF. In contrast, Marsh and Drabkin[6] found no inhibition of liver RNA or DNA synthesis by normal rats after a single injection of *N*-hydroxy-AAF. They did find, however, that a single dose of *N*-hydroxy-AAF given 1 hr after partial hepatectomy inhibited RNA and DNA synthesis approximately 85 and 95 per cent, respectively, when measured 24 hr postoperatively. Marsh and Drabkin[6] suggested that the difference between their results and those of Zieve and Gutmann[4] was due to differences in the rate of absorption of the carcinogen in the two studies, since different solvents were used in administering the *N*-hydroxy-AAF. Levels of *N*-hydroxy-AAF in liver and plasma were found to be twice as high when the carcinogen was administered as a solution in propylene glycol, as used by Zieve and Gutmann,[4] when compared to the same dose given as a suspension in 1% gum acacia.[6]

In an attempt to resolve this discrepancy, we have examined the effects of *N*-hydroxy-AAF on RNA and DNA synthesis in liver slices from normal and partially hepatectomized male Holtzman rats. Metabolism of *N*-hydroxy-AAF by liver slices appears to be similar, if not identical, to that of liver cells *in vivo*.* Use of the slice obviates problems of rapid urinary and biliary excretion of the carcinogen as well as metabolism of the carcinogen by the flora of the digestive tract.[7]

* C. D. Jackson and C. C. Irving, unpublished work.

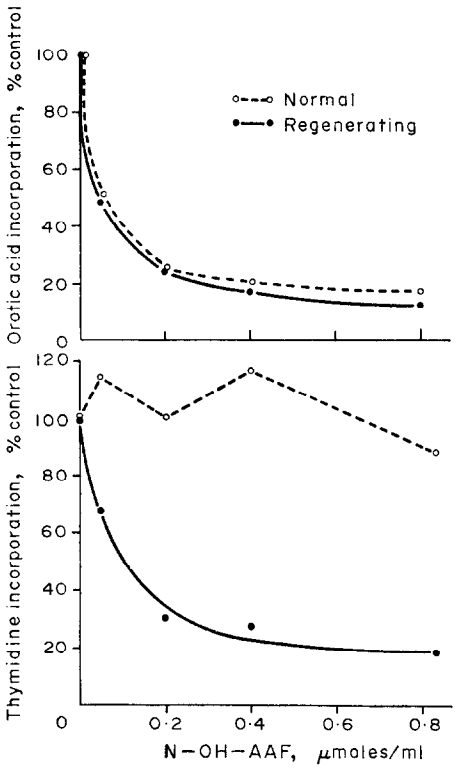

FIG. 1. Effect of *N*-hydroxy-AAF on RNA synthesis (upper) and DNA synthesis (lower) in liver slices from normal (○- - -○) and regenerating (●——●) liver. Liver slices were incubated 2 hr in Krebs–Ringer phosphate buffer in the presence of *N*-hydroxy-AAF at the concentrations shown. Incorporation of orotic acid-5-^{3}H into RNA and thymidine-methyl-^{3}H into DNA was determined as described in the text and expressed as per cent incorporation by slices in the absence of *N*-hydroxy-AAF. Each value is the average of two samples; the maximum variation between duplicate samples was ± 3 per cent of the mean. Actual values for control slices were: normal liver, RNA, 3,300 dis/min/A_{260} unit; DNA, 261 dis/min/μg; regenerating liver, RNA, 13,000 dis/min/A_{260} unit; DNA, 1,620 dis/min/μg.

Partial hepatectomies were performed under ether anesthesia as described by Higgins and Anderson.[8] Livers were removed 18–20 hr after partial hepatectomy and slices were cut with a Stadie–Riggs tissue slicer and placed in a small volume of Krebs–Ringer phosphate buffer[9] with calcium ion omitted. The first and last slices of each lobe were discarded. Each sample, consisting of several slices of liver, was incubated in 50-ml beakers containing 2 ml of Krebs–Ringer phosphate buffer previously equilibrated at 37° with oxygen. The incubation mixture contained either orotic acid-5-^{3}H (5 μCi/ml) or thymidine-methyl-^{3}H (25 μCi/ml) and *N*-hydroxy-AAF at concentrations indicated in Fig. 1. After incubation for 2 hr, the slices were removed and rinsed in 20 ml of 0·9% NaCl, then frozen on dry ice. RNA and DNA were extracted from the liver slices and their specific radioactivities were determined as previously described for whole liver.[10] Results were normalized by expressing the data as per cent of the incorporation by slices in the absence of *N*-hydroxy-AAF. The studies on the incorporation of orotic acid into RNA, shown in the upper half of Fig. 1, clearly demonstrate that *N*-hydroxy-AAF is equally inhibitory to RNA synthesis in both normal and regenerating liver. In contrast, the effect of the carcinogen on DNA synthesis was markedly different in normal and regenerating liver (Fig. 1, lower half). Inhibition of DNA synthesis in slices from regenerating liver was found to parallel that of RNA synthesis with a concentration of 0·2 mM *N*-hydroxy-AAF producing 70–80 per cent inhibition. On the other hand, DNA synthesis in normal liver slices was not significantly inhibited at any concentration of *N*-hydroxy-AAF studied.

Several explanations for the differential effects of *N*-hydroxy-AAF on DNA synthesis in norma

and regenerating liver are possible: (1) the carcinogen might interfere with regulatory mechanisms which initiate DNA synthesis and cell division in regenerating liver;[6] (2) the incorporation of thymidine-^{3}H into DNA of normal liver may represent repair synthesis which is not inhibited by *N*-hydroxy-AAF, while the incorporation of thymidine-^{3}H into DNA of regenerating liver does in fact represent DNA replication, which is inhibited by the carcinogen; and (3) the incorporation of thymidine-^{3}H into DNA in normal liver is not due to DNA replication in differentiated hepatocytes but in small nondescript cells in portal triads which could conceivably be stem cells, hepatocyte precursor cells, connective tissue cells, bile duct precursor cells, etc. The inhibitory effect of *N*-hydroxy-AAF probably requires formation of AAF-N-sulfate by a sulfotransferase;[10] this enzyme may be present only in mature hepatocytes. On this basis, DNA synthesis in normal liver would not be inhibited by the carcinogen. In contrast, in regenerating liver when the bulk of DNA synthesis is occurring in differentiated hepatocytes, one would expect these cells to show the inhibitory effect.

Although we cannot eliminate further consideration of the first two possibilities, there are a number of observations which are consistent with the third alternative. Female Sprague–Dawley rats are quite resistant to the hepatotoxic and hepatocarcinogenic effects of AAF.[10] Administration of 0·04% AAF in the diet had no effect on the low rate of liver DNA synthesis or mitotic index in normal male or female rats during the first 3 weeks.[10] In male rats at the end of 3 weeks, a population of liver cells resistant to the carcinogen begins to proliferate and eventually forms hyperplastic nodules.[10] In a second study, we found that oral administration of AAF markedly inhibited liver regeneration in female rats.[11] Both the increased DNA synthesis and mitotic index expected after partial hepatectomy were greatly reduced. However, a slow but continued increase in liver mitotic index of the AAF-treated female, partially hepatectomized rats indicated the presence of cells which were resistant to the carcinogen and which responded to the proliferative stimulus of partial hepatectomy. The resistant cells continued to proliferate in the presence of the carcinogen and, as observed in normal male rats, progressed to hyperplastic nodules.[11]

The observation that *N*-hydroxy-AAF inhibited RNA synthesis in both normal and regenerating liver is consistent with the fact that all liver cells are actively synthesizing RNA under either condition.

It has been suggested that the production of a "resistant" cell line is one of the initial effects of a chemical carcinogen.[12] If a small population of precursor cells for differentiated hepatocytes exists which have a low *N*-hydroxy-AAF sulfotransferase activity and thus are resistant to the toxic effects of AAF and its *N*-hydroxy metabolite, a mechanism for the establishment of a resistant cell line can be postulated as follows: upon continuous administration of AAF (or *N*-hydroxy-AAF), differentiated hepatocytes are killed, leading to a compensatory proliferation of these precursor cells, some of which eventually give rise to hyperplastic nodules and then hepatocellular carcinoma.

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