LIVER DEGENERATION AND CIRRHOSIS PRODUCED BY
1: 2: 5: 6–DIBENZANTHRACENE

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Coal tars and shale oils have been used extensively to induce cancer in mice, rats, rabbits, and fowl. The mortality among the animals treated is high, and an intense tissue reaction is usually produced at the site of contact. Davidson (1) showed that external application of tar to the ears of rabbits, or injection of an ether solution would produce a degeneration of the liver cells and cirrhosis. Polson (2) obtained the same effect by the use of shale oil. Since the materials employed were complex mixtures, it remained undetermined whether the carcinogenic power and the toxicity on the liver were associated properties of the same chemical. In the present study rabbits were treated with 1: 2: 5: 6–dibenzanthracene, a substance which has been repeatedly demonstrated as a powerful carcinogenic agent. The subcutaneous injection of the chemical was followed by extensive damage to the liver parenchyma in many of the animals treated. This note is a report of the results obtained with the crude and the purified hydrocarbon.
In the first series of experiments, rabbits of various breeds were injected subcutaneously with a 0.4 per cent solution of commercial \( 1: 2: 5: 6 \)-dibenzanthracene in lard.\(^1\) The injections, each of 10 to 15 mg. of the chemical, were made at weekly intervals for a first group of 3 rabbits, at monthly intervals for the other groups, with occasionally two to three month intervals for the rabbits surviving more than six months. Twelve out of a total of 18 rabbits died with signs either of acute liver necrosis or of chronic liver degeneration and cirrhosis. Of this group, 83.3 per cent suffered from ascites and edema in varying degree at the time of death. Individual resistance varied greatly, 6 rabbits living more than two and a half years, during which time they received large amounts of the hydrocarbon without any apparent ill effect, as shown by a steady increase in body weight. During this period one of these rabbits received 275.7 mg. dibenzanthracene in 16 injections, or approximately 1.0 mg. per 15 gm. body weight. At autopsy, the liver showed fatty degeneration, but no fibrosis.

Six control rabbits, injected with lard alone instead of the dibenzanthracene solution, were kept for eighteen months under the same conditions as the experimental animals. During this period, the average body weight in-

\(^1\) The hydrocarbon was obtained from the Eastman Kodak Company.
creased from 2.8 to 3.9 kg. When killed, these animals were in excellent health and no lesions were found at autopsy.

Commercial dibenzanthracene is contaminated by a colored impurity which is not removed by recrystallization. In order to determine whether the effect on the liver was caused by the hydrocarbon itself, crude dibenzanthracene was purified by repeated washing of a toluene solution with concentrated sulphuric acid and further recrystallization of the substance until a colorless product was obtained. In the next experiment 4 rabbits were treated with a colloidal solution of this purified dibenzanthracene (3). A total of 11 injections were made at weekly intervals, each injection being adjusted to represent 5 mg. hydrocarbon per kilo of body weight. Three rabbits died six, twenty-one, and twenty-nine days following the last injection.

At autopsy important degenerative changes of the liver were found, along with edema of the skin and transudates in the peritoneal, pleural and pericardial cavities. The fourth rabbit was killed to end the experiment. There was no ascites, but there was a marked necrosis of the liver cells and congestion of the organ. About one-fifth of the total amount of dibenzanthracene injected was recovered by benzene extraction of the dried skin of the rabbits treated. The same method failed to demonstrate the presence of dibenzanthracene in the benzene extracts of the liver, the bile or the ascitic fluid.

The carcinogenic power of each batch of dibenzanthracene employed was tested in mice, 70 to 80 per cent of which died with sarcoma within a year.

Microscopically the lesions produced in the rabbit liver by the crude or purified dibenzanthracene were those of acute necrosis when the injections

FIG. 3. RABBIT 6, INJECTED SUBCUTANEOUSLY WITH A COLLOIDAL SUSPENSION OF PURIFIED DIBENZANTHRACENE: INOCULUM, SIX WEEKS AFTER INJECTION, SHOWING CONGESTION AND PETECHIAE IN THE UNDERLYING MUSCLE SHEET
Other lesions or reaction practically absent.
were frequent, of cirrhosis with intermediate stages of degeneration and regeneration when time was allowed for the liver to recuperate between the injections. Massive destruction of liver cells occurred in the central zone of the lobules, with practically no transition between the necrotic area and the apparently normal cells surrounding the portal tracts (Fig. 1). In the necrotic areas, the liver cells appeared opaque and uniformly stained with eosin, the nuclei being absent or represented by pyknotic débris. Vasodilatation and congestion were frequent in the degenerated liver tissue, the necrotic areas being occupied by engorged blood vessels (Fig. 2). Passive distention of the central veins in the affected areas appeared to result from degeneration of the liver cells and subsequent removal of the necrotic material. When necrosis was more extensive and involved confluent lobules, hemorrhages occurred in the affected region and on the surface of the liver, which was covered with a thick layer of fibrin. When recovery occurred, there was produced the typical abnormal lobular architecture seen in toxic cirrhosis, with interstitial fibrosis and proliferation of the bile-ducts. These results suggest that the hydrocarbon, when introduced subcutaneously, may be eliminated through the liver, although no dibenzanthracene could be demonstrated in the liver or the bile extracts. Other lesions presented by the rabbits treated were degeneration of the parenchyma of the lymph nodes and the spleen. The spleen showed intense engorgement of the pulp sinuses, hemorrhage into the malpighian follicles and punctate necrosis of lymphocytes. Hemorrhage may have been the cause of a marked pressure atrophy of malpighian bodies. Much pigment was often present, as seen in severe hemolytic anemia. Lymph nodes showed lesions similar to those encountered in the spleen. At the site of inoculation the most obvious changes were on the blood vessels, as shown by vasodilatation and petechiae (Fig. 3).

**References**

1. **Davidson, J.** J. Path. & Bact. 28: 621, 1925.

These results are in agreement with those of Chalmers and Peacock (4) who found that dibenzanthracene injected intravenously disappeared rapidly from the blood but its fate, beyond the liver, could not be ascertained.