Production of Sarcoma in Rats with Light Green SF

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In 1937 Schiller (1) reported that the dye, light green SF, was carcinogenic for rats but not for mice. In his experiment, 7 rats (white, and spotted black and white) received, once or twice weekly, subcutaneous injections of 1 to 3 cc. of an aqueous 2 per cent, and ultimately a 3 per cent, solution of the dye. When the animal's condition deteriorated, the injections were omitted or the volume was diminished. After about a year, 4 of the rats developed sarcomas at the site of injection.

As Schiller's experiment was conducted with a small number of heterogeneous animals, and since no confirmation had appeared in print, it seemed desirable to repeat Schiller's experiments, using a sample of light green SF supplied by Coleman and Bell.

METHODS

Our rats were obtained from a local breeder and were descended from the Wistar strain. Injections were made subcutaneously over the abdomen as nearly as possible in the same region every time. The first injection consisted of 2 cc. of a 2 per cent aqueous solution of light green SF, but this prostrated the animals, and the volume of subsequent doses was reduced to 1 cc. Injections were usually given twice weekly. After 1 month (9 treatments), the concentration of the dye solution was increased to 3 per cent, and following this increase the animals' condition at times became so poor that it was deemed advisable to omit a scheduled treatment.

As a result of the repeated injections, there was a gradual increase in subcutaneous fibrous tissue which ultimately made injection more difficult. Circumstances forced the discontinuation of injection after the 33rd week. During this time, each rat received 59 doses, or a total of 1.7 gm. of dye. In order to minimize leakage of the inoculum, injections were made with 24 or 25 gauge needles, but in spite of this, some leakage always occurred, so that none of the animals retained all of the amount administered.

RESULTS

Soon after completion of an injection the rats turned diffusely green, but as the dye was eliminated by the kidneys, their color returned to normal. However, the subcutaneous tissues about the site of injection soon became deeply stained, and some green color persisted for many months after cessation of treatment. The kidneys, too, became deeply pigmented. The dye was deposited in cells of the convoluted tubules and persisted for several months after treatment was stopped, but ultimately was eliminated.

The experiment was begun with 30 rats, but 4 died within 8 weeks, and 2 more died within 19 weeks after treatment was started. Fifteen rats developed tumors at the site of inoculation at the following intervals after the first injection: 35, 36, 37, 43, 44, 44, 48, 51, 56, 57, 59, 66, 83, 84, and 85 weeks. Nine rats died tumor-free 45, 57, 59, 61, 67, 71, 81, 85, and 92 weeks after the first injection. Hence, tumors developed in 63 per cent of the effective total of 24 rats.

Some tumors were easily separated from both skin and abdominal muscle; others were closely adherent to both structures, and still others were adherent to the skin only. Three of the last-mentioned were associated with slight ulceration of the skin. All tumors were fairly well circumscribed and rounded, and consisted of slightly translucent, whorled gray tissue. Most of the tumors grew rapidly after they became manifest. Microscopically, the tumors were fairly uniform and consisted of fusiform cells arranged in whorls and interlacing bundles. Mitoses were numerous in most tumors, and in only a few were giant cells at all common, although nearly all tumors contained some. Although some cells in a few of the tumors superficially resembled smooth muscle cells, no myofibrillae could be demonstrated, and all tumors were classed as fibrosarcoma. Typical examples of the tumors are shown in Figs. 1 and 2.

DISCUSSION

The question whether the tumors were produced by the dye or by some impurity is pertinent. Unfor-
Unfortunately, we cannot answer this question. An inquiry to Coleman and Bell as to the purity of the dye and the identity of impurities elicited the following reply, "We purchase this dye commercially and purify it for use as a biological stain... The dye is a very complicated organic compound and we doubt if it could be prepared in a purity comparable with C.P. chemicals. It is reasonable to believe that it contains other organic compounds as impurities. What these compounds are and how much we are unable to say." The complexity of the structural formula of light green as given by Conn (2) is shown by Fig. 3.

It seems probable that a higher incidence of tumors would have been obtained in our experiment if administration of the dye had been continued longer. However, the development of sarcomas in nearly two-thirds of the effective total number of rats treated indicates that, under proper conditions, the dye is a potent carcinogen.

SUMMARY

The reported carcinogenicity of a commercial sample of the dye, light green SF, administered by subcutaneous injection is confirmed. Sarcomas ultimately developed at the site of injection in 15 of 24 rats that received a total of 1.7 gm. of the dye in aqueous solution over a period of 33 weeks, but it is uncertain whether the carcinogenic action is attributable to the dye or to an impurity.

REFERENCES

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