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IT has been recently found that when 2-acetylaminofluorene (AAF) and several other carcinogenic amines are administered to rats, *N*-hydroxylation of the compound occurs *in vivo*[1,2]. Using AAF, or several other amines, it has been shown that the *N*-hydroxy metabolite (N—OH—AAF) was a more active carcinogen than the parent compound[3].

It was therefore proposed that N—OH—AAF may be the proximate carcinogen when AAF is administered to rats[4]. In the course of some preliminary experiments we have obtained additional evidence for this proposition.

Ten 8-week-old male MRC rats (210 g), were injected once subcutaneously on the back with 5 mg of N—OH—AAF dissolved in 0·5 ml. of warm olive oil. A week later a shaved area of interscapular skin was painted with a 2 per cent solution of N—OH—AAF in acetone, using a fine hair brush. The rats were painted at first three times, and later five times a week until the fortieth week, when they had been treated 125 times. The animals were given powdered laboratory chow and as much water as they wanted to drink. Once a week they were weighed and carefully inspected. At the twentieth week signs of skin irritation and exudation appeared, and by the thirty-third week ulcers were present on most of the animals. At the thirty-fifth week a crop of skin tumours suddenly arose, and their progress was recorded weekly on graph paper maps. One rat died of pneumonia at 25 weeks without any other lesions, and the remaining nine rats were killed when their tumours caused a decline in health.

A total of ten subcutaneous tumours occurred near the site of the initial injection in seven of the nine surviving rats. Eight of these were invasive fibrosarcomata, and two were well differentiated, relatively benign, fibrous tumours. Most of the subcutaneous tumours were a little distant from the original site of injection, no doubt as a result of migration of the oily solution. The latent period for the subcutaneous tumours varied from 20 to 44 weeks, with a mean latency of 29·6 weeks.

The earliest skin tumour in the painted area was found in the twenty-first week, and by the thirty-seventh week all the surviving rats had tumours in the painted areas. Six out of nine rats survived longer than 35 weeks, and had a total of sixty skin tumours. The number of skin tumours on each rat increased steadily from the twenty-fifth to the fifty-second week, when the final animal was killed. Only four of the original sixty tumours regressed. Of the fifty-six tumours which persisted, twenty (36 per cent) showed a steady increase in size, and were found on

histological examination to be invasive carcinomata. The majority of the carcinomata were of the squamous type, but a few were basal-cell carcinomata. The morphology of the benign tumours was similar to that described for mouse skin tumours[5]. Six rats had eight primary tumours which arose at a distance from the painted areas, namely in the Zymbal glands, breast, and lungs. One of the Zymbal tumours and one of the breast tumours metastasized widely.

The induction of malignant skin tumours in rats by painting with N—OH—AAF was striking in view of the usual refractoriness of rat skin to chemical carcinogens. In rats painted with the parent compounds 2-AAF and 2-AF, skin tumours are very rare, and nearly always occur at a distance from the painted area. The present results, therefore, provide strong evidence for the local action of N—OH—AAF as a direct carcinogen for rat skin and subcutaneous tissue, without metabolic modification. It is considered that the single subcutaneous injection at the beginning of the experiment could not have much influenced the response of the skin. The experiments are being repeated and skin tumours have already been obtained by painting alone. The occurrence of sarcomata at or near the site of injection is also of interest in that they appear to have been caused by a single injection, whereas in work reported previously, multiple injections were given[6]. The fact that combined treatment was given complicates this interpretation, however, because the occurrence of distant neoplasms indicates that there must have been significant absorption either of N—OH—AAF or of its parent amines. Furthermore, it is impossible, at present, to state what influence the painting may have had in the appearance of the subcutaneous tumours.

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