THE DEVELOPMENT OF MALIGNANT TUMOURS OF MOUSE SKIN AFTER "INITIATING" AND "PROMOTING" STIMULI

II. THE INFLUENCE OF ALTERNATE APPLICATIONS OF CROTON OIL ON MALIGNANT TUMOUR-PRODUCTION BY REPEATED APPLICATIONS OF DILUTE 9,10-DIMETHYL- 1,2-BENZANTHRACENE (DMBA)

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SHUBIK (1950) found that a single application of 9,10-dimethyl-1,2-benzanthracene (DMBA) followed by repeated applications of croton oil gave rise to more benign and less malignant tumours than did repeated applications of DMBA. One of the several possible explanations of the different incidence of malignant tumours in these two treatment groups is that croton oil inhibited the production of malignant tumours by DMBA. This possibility is mentioned by Foulds (1954), who reviews the evidence for stages in carcinogenesis, and for what he has called "tumour-progression", in mouse skin and other tissues. The experiment to be described in this paper was intended to test this possibility.

MATERIALS AND METHODS

The materials and methods used were essentially the same as those described in the first paper of this series (Roe, 1956*a*).

EXPERIMENTAL

Thirty-nine male albino mice of the "S" strain, aged 7 to 9 weeks, after successful vaccination on the tail, were divided at random into two groups of 19 and 20 (Groups 1 and 2 respectively).

Both groups were given a single application of 0.2 ml. 0.15 per cent DMBA in acetone to the clipped skin of the back. Beginning 26 days later, both groups were given a course of 13 weekly applications of 0.3 ml. 0.03 per cent DMBA in acetone. Mice of Group 2 were given, in addition, a course of 21 weekly applications of 0.3 ml. 0.5 per cent croton oil ("Batch I" croton oil was used, see Roe, 1956*b*) in acetone beginning on the 22nd day of the experiment: applications of croton oil and of the dilute DMBA alternated at 3- to 4-day intervals until the last application of the latter had been given.

The first malignant tumours to appear in individual mice were, whenever possible, removed by biopsy under ether anaesthesia. The site of scars resulting from biopsy were noted (see Roe, 1956*a*; p. 64).

RESULTS

Incidence of malignant tumours

The first malignant tumour appeared in Group 2 on the 96th day. One mouse in Group 1 died before the 96th day and was excluded from the results, leaving for consideration 18 mice in Group 1 and 20 in Group 2. The experiment was terminated, and all surviving mice killed, on the 163rd day; but several mice died, or had to be killed because of multiple malignant skin tumours, before this date. The average survival time of the 18 mice in Group 1 was 152 days, and that of the 20 mice in Group 2, 151 days.

Ten of the 18 mice in Group 1, and 18 of the 20 in Group 2, bore malignant tumours of the skin. Altogether 26 such tumours were seen in Group 1 and 38 in Group 2; all these were confirmed histologically, using the criterion of penetration of the panniculus carnosus. In addition, 6 "probably malignant" tumours (see Roe, 1956a, p.66, for definition) were seen in Group 1, and 8 in Group 2. Metastases were seen in the regional lymph nodes of two mice in Group 2. The highest number of definitely malignant tumours seen in an individual mouse of either group was six. Eight mice in Group 1 and 10 in Group 2 had their first malignant tumours removed at biopsy.

The average latent interval between the beginning of treatment and the appearance of malignant tumours was approximately the same for both groups (130.3 days in Group 1, and 134 days in Group 2).

A significance test for the difference between the mean numbers of malignant tumours in Group 1 and in Group 2, gives $t = .79$, on 36 degrees of freedom, a value which could be exceeded by chance with a probability of $P = 0.5$.

CONCLUSION

The incidence of malignant tumours in mice treated repeatedly with DMBA was not diminished by concurrent croton oil treatment; on the contrary it was slightly but not significantly increased. The average latent interval of induction of malignant tumours was unaltered by croton oil treatment.

SUMMARY

1. Two groups of mice were treated with one application of 0.15 per cent DMBA followed, after a 26-day interval, by a course of 13 weekly applications of 0.03 per cent DMBA. One group received in addition 21 weekly applications of 0.5 per cent croton oil, alternating at first with those of the dilute DMBA.

2. The incidence of malignant tumors was higher in the group which received croton oil treatment, though not significantly so.

3. This result gives no support to the suggestion that croton oil may inhibit the induction of malignant tumours in mouse-skin by DMBA.

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