Copper Acetate as an Accelerator in Mouse Skin Carcinogenesis by 9,10-Dimethyl-1,2-benzanthracene

In a previous paper, it was shown by FARE[1] that mice painted with 0.05% 9,10-dimethyl-1,2-benzanthracene (DMBA, new nomenclature 7,12-dimethyl-benz-(a)-anthracene) + 0.15% cupric oxyacetate hexahydrate (CuAc) gave a significantly higher tumour incidence than did 0.05% DMBA alone, when acetone was used as the solvent.

The experiments described here were carried out to enquire into possible mechanisms for this enhancement, and in particular to ascertain whether CuAc in acetone was a carcinogen per se and whether it had any 'initiating' or 'promoting' action, as generally understood by the terms (e.g. as used by WALPOLE[2] and SALAMAN and ROE[3]), on DMBA carcinogenesis.

Plan of experiments. 325 male white mice were used, aged 10–14 weeks, taken from our out-bred stock in a closed colony. Females were not used because of their predisposition to breast cancer, particularly when treated with DMBA. The mice were randomized by a two-stage process, firstly by human selection from a pool and then by blind selection using a mechanical device, and were housed in boxes of five. Proprietary cube feed and drinking water were available ad libitum.

Analar acetone (Hopkin and Williams) was redistilled, and DMBA (Eastman Kodak), croton oil (BPC) and CuAc (Hopkin and Williams) were used without purification at concentrations of 0.05, 0.2, and 0.15% respectively in the redistilled acetone. Twice weekly applications of 0.2 ml between the scapulae were applied to the seven groups shown in the Table. At no time was more than one chemical applied simultaneously in the acetone. Croton oil was used as a standard 'promoting' agent.

Ten mice from each group were killed in pairs at various times as appropriate, but always midway between the two weekly paintings. Thus a pair killed after '3 weeks' would have received 5 applications. Dorsal skin within the painting area was removed and protein bound copper and hydrocarbon were determined as described by FARE. The mice to be killed at any one time were again selected by a random technique.

The remaining mice in each group were used to assess the carcinogenicities of the various treatments. They were considered to be 'tumour-bearing' or 'normal', and no account was taken of the several other parameters such as the number of tumours produced, their histological appearance, size, rate of growth, anatomical distribution etc.

Carcinogenicity of CuAc (Experiment 1). No tumours were found in the mice painted throughout with copper acetate when the survivors were killed after 70 weeks treatment. Protein bound copper rose to 85 μg/g protein nitrogen when the third pair were killed for assay after 8 weeks' treatment, and it remained at this level for the remainder of the experiment.

Promoting activity (Experiments 2–4). After an initial treatment with DMBA, four 100 μg applications, treatment with CuAc did not give any greater tumour incidence than did painting with acetone. By the seventieth week, 7 mice out of 40 had developed tumours (acetone treated) and 8 out of 40 (CuAc treated). Croton oil gave enhanced tumour production, 17 out of 40, and the χ^2 test showed significance at the 5% level. Skin protein bound copper, measured at the start of and after 1, 2, 3, and 4 weeks' copper treatment, gave comparable values to those obtained from the first two pairs of mice killed in experiment 1. In other words, pretreatment with DMBA did not affect the rate of copper binding when CuAc was given subsequently. There were no differences between groups 2, 3, and 4 as regards mice which died without producing tumours (8, 6, and 7 mice respectively).

Bound hydrocarbon (measured fluorimetrically, and expressed as DMBA) is given in Figure 1. There was clearly no difference in the rate of decay of skin protein bound hydrocarbon, and the tumour incidences obtained in these three experiments do not therefore appear to correlate with the persistence of protein bound hydrocarbon in the skin.

The croton oil experiment did indicate, however, that the mice used in our experiments responded to a known 'promoting' agent in the expected manner.

Initiating activity (Experiments 5–7). Mice painted with the copper solution for 10 weeks and then with DMBA gave a tumour yield which was higher than that which

The various treatments

Experiment	No. of mice	Treatment
1	45	CuAc throughout
2	50	2 weeks' DMBA, then CuAc
3	50	2 weeks' DMBA, then croton oil
4	50	2 weeks' DMBA, then acetone
5	45	10 weeks' CuAc, then DMBA
6	45	10 weeks' CuAc, 5 weeks' acetone, then DMBA
7	40	10 weeks' acetone, then DMBA

Experiment 1 was a carcinogenicity test, experiments 2–4 'promotion' tests, and experiments 5–7 'initiation' tests. All surviving mice were killed after 70 weeks' treatment.

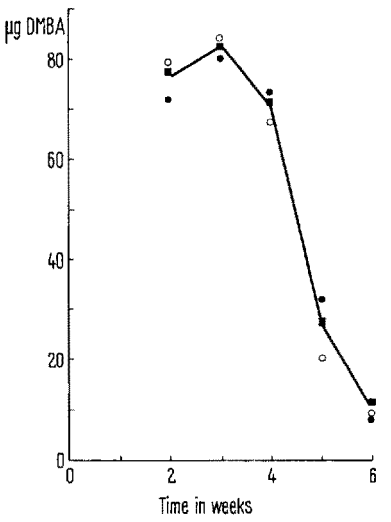

Fig. 1. Skin protein bound hydrocarbon, expressed as μg DMBA per 100 mg nitrogen, resulting from treatment with DMBA for 2 weeks followed by CuAc (o), croton oil (■), and acetone (●).

[1] G. FARE, Brit. J. Cancer *18*, 768 (1964).
[2] A. L. WALPOLE, Ciba Foundation Symposium on Carcinogenesis, 41 (1959).
[3] M. H. SALAMAN and F. J. C. ROE, Brit. Med. Bull. *20*, 139 (1964).

resulted when acetone was painted for 10 weeks followed by the carcinogen. The number of tumour-bearing mice after 12 weeks' hydrocarbon application was 18 out of 35 (copper pretreatment) and 8 out of 30 (acetone pretreatment). The χ^2 test showed significance at the 5% level. When the copper pretreatment was followed by 5 weeks' acetone treatment before the hydrocarbon was applied, there was no such enhancement of tumour yield (13 out of 35 after 12 weeks' DMBA treatment, not significant at the 5% level). Assays showed that the decrease in protein bound copper in the skin after the initial CuAc treatment was independent of subsequent treatment, whether with DMBA directly or by DMBA after an intervening period of acetone. Hydrocarbon binding occurred more readily after the intervening acetone treatment than it did when DMBA applications followed directly after the copper treatment (Figure 2). It will be seen from this Figure that in experiment 6, a single application of DMBA sufficed to give a virtually identical value to that resulting from three applications under the conditions of experiment 5.

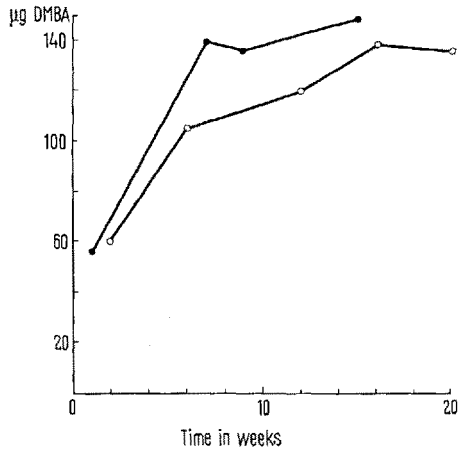

Fig. 2. Skin protein bound hydrocarbon, expressed as µg DMBA per 100 mg nitrogen, resulting from treatment with DMBA following treatment for 10 weeks with CuAc (o) and 10 weeks CuAc then 5 weeks acetone (●). Abscissa zero is the start of DMBA applications.

Conclusions. Copper acetate in acetone solution is not carcinogenic under the test conditions, nor is it a potentiating or an initiating agent. It only gives increased tumour yields when it is applied at the same time as the carcinogen (FARE[1]) or when the two chemicals are preser in the skin at the same time as in experiment 5.

When the hydrocarbon was given first (experiment 2) there was no potentiation although these conditions were met. This may be explained by suggesting that there may have been an insufficiency of hydrocarbon present during the copper applications or that there was a sufficient amount that did not persist for a sufficient time.

SALAMAN[4] advocated the use of croton oil for detecting the action of substances weakly carcinogenic or non-carcinogenic for the skin. In an attempt to confirm the findings that copper acetate was not carcinogenic, and to check that the enhanced tumour production in experiment 5 was not due to a slight carcinogenicity of CuAc, 20 mice were treated with CuAc solution for 10 weeks and then with croton oil solution for 60 weeks. Three tumours appeared in two animals whilst three mice from a control group of 20 treated with croton oil throughout developed tumours. This experiment therefore confirmed the non-carcinogenicity of copper acetate[5].

Résumé. Une solution de l'oxyacétate de cuivre n'est pas cancérigène pour la peau de la souris blanche. L'acétate n'est ni un 'initiator' ni un 'promoter' pour la cancérisation par 9,10-diméthyl-1,2-benzanthracène. Cependant, le traitement des animaux avec l'acétate pendant 10 semaines puis avec l'hydrocarbure augmente la production des tumeurs.

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[4] M. H. SALAMAN, Ciba Foundation Symposium on Carcinogenesis (1959), p. 70.

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PRO EXPERIMENTIS

Purification chromatographique d'une préparation de thymus douée d'activité hormonale

En son temps une méthode d'extraction a été décrite qui permet d'obtenir du thymus de veau une préparation[1,2] capable de supprimer les conséquences de la thymectomie chez le cobaye et le rat. On décrira ici une méthode de purification chromatographique de cette préparation.

L'activité biologique a été estimée à l'aide d'une méthode[3,4] basée sur la propriété de l'extrait de thymus de supprimer la stimulation de la créatinurie par la thyroxine chez les cobayes thymi-thyréoprives castrés. Ceux-ci éliminent spontanément 0,5–0,7 mg de créatine/100 g/24 h; 24 h après une injection de 10 µg de thyroxine par 100 g de poids vif de l'animal, le taux d'excrétion s'élève à 2,0–3,5 mg/100 g/24 h. On considère que l'effet de la thyroxine est supprimé par l'injection simultanée d'extrait de thymus si le taux moyen d'excrétion correspond à celui des castrats thymi-thyreoprives non traités chez

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[4] J. COMSA, Am. J. Physiol. *166*, 550 (1951).