

# The Apparent Anticarcinogenic Action of Lanolin

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In the course of their work on the carcinogenic action of oils and tars on mouse's skin, Twort and Twort (4,5) observed that addition of anhydrous lanolin often led to a reduction in neoplastic response. The inhibitory effect was greater than that obtained with benzene as diluent, and also occurred when lanolin was applied separately to the skin during the intervals between paintings. In view of this apparently specific inhibition of carcinogenesis, the use of lanolin was subsequently recommended as a preventive against occupational cancer in the mule-spinning industry (6).

A more pronounced inhibition of skin carcinogenesis by anhydrous lanolin was recently reported by Simpson, Carruthers and Cramer (1,2,3) in connection with methylcholanthrene. They found that a 0.3 per cent solution of this carcinogen in lanolin, applied thrice weekly for 14 weeks, was almost completely ineffective in inducing tumors or even in initiating those preliminary changes (epilation, destruction of sebaceous glands, epithelial hyperplasia etc.) which are regularly observed when the carcinogen is applied in benzene. This inhibitory effect was shown not to result from (a) inactivation of the carcinogen by the lanolin (b) the prevention of the carcinogen from reaching the epithelial cells, or (c) failure of the carcinogen to persist long enough *in situ*. Since the amount of methylcholanthrene applied was calculated to be adequate for carcinogenesis, and since the skin treated with the lanolin solution of the carcinogen, far from becoming resistant, was actually more responsive to subsequent painting with a solution of the carcinogen in benzene (3), they put forward the tentative hypothesis that "unaltered methylcholanthrene is a sensitizing agent, itself non-carcinogenic, that prepares the skin for subsequent action by metabolic derivatives of the carcinogen, or by substances formed in the tissues as a result of exposure to these metabolic products," with the implication that lanolin specifically interferes with this mechanism.

Irrespective of the possible merits of this hypo-

thesis, acceptance of any specificity of action on the part of lanolin would be unjustified until simpler interpretations (based on such physical factors as a sub-threshold concentration of the carcinogen in the tissues) are definitely excluded.

When a benzene solution of methylcholanthrene is applied to the skin the benzene evaporates immediately leaving behind the carcinogen dissolved in the minute amounts of sebum normally present on the skin. The effective concentration of the carcinogen will therefore be much higher than the original concentration in the benzene. But when using a non-volatile solvent such as lanolin, the effective concentration practically corresponds to that of the solution as originally applied. It is hardly to be expected that two solutions of a carcinogen, one in a volatile and the other in a nonvolatile solvent, will elicit the same response, even though the amount of carcinogen applied and the original concentration are the same in both cases.<sup>1</sup>

Assuming that the inhibitory action of lanolin were entirely due to this dilution effect, one should expect to find (i) that with higher concentration, of the carcinogen in lanolin the neoplastic response should approximate that obtained in benzene, (ii) that other non-volatile solvents would behave similarly to lanolin, and (iii) that the effect should also be demonstrable with carcinogens other than methylcholanthrene.

The following experiments were undertaken to test these three possibilities.

<sup>1</sup> A simple empirical confirmation of this was obtained by determining the relative concentrations of an irritant in a volatile and non-volatile solvent, respectively, required to produce comparable degrees of irritation in the mouse's skin. Using croton oil as an irritant, it was found that a 3.0 per cent solution in liquid paraffin produced approximately the same degree of change in the skin as a 0.5 per cent in acetone, thus indicating a six-fold increase in the effective concentration in the latter. The exact ratio will, of course, depend on the amount applied in the case of the volatile solvent (though not, presumably, in the case of the nonvolatile solvent.).

## EXPERIMENTAL

The animals used for the work were white mice bred in this laboratory over a period of many years, representing a fairly homogeneous, though not genetically pure, strain. Twenty-five mice were used for each of the 9 experimental groups, applications being made to a small area of skin in the interscapular region, employing a capillary pipette for solutions in benzene, and a small glass spatula for the preparations in lanolin or liquid paraffin. Prior to each application, the hair was clipped with scissors, thus obviating the use of a chemical epilator.

The methylcholanthrene was made up as a 0.3 per cent solution in benzene, and as 0.3 per cent and 3.0 per cent solutions, respectively, in anhydrous lanolin (groups I-III, Table I). These were applied twice weekly until all the surviving animals had developed tumors after which the animals were left untreated until they died. The other carcinogen used in this investigation, 9,10-dimethyl-1,2-benzanthracene, being more potent than methylcholanthrene was applied only once weekly, in the following concentrations: 0.1 per cent in benzene, lanolin, and liquid paraffin, respectively, and 2.0 per cent in lanolin and liquid paraffin, respectively, treatment being continued until all the survivors had developed tumors. Careful note was made, at half-weekly intervals, of the survival rate in each group, and of the development of tumors (benign and malignant), malignancy being subsequently confirmed by histological examination.

“the average response”) was 12½ weeks with 0.3 per cent in benzene, but 28 weeks with the same concentration in lanolin; while the total tumor yield was also greatly reduced in the latter (see groups I and II, Table I). However, with a 3.0 per cent solution in lanolin, the average response was, if anything, earlier than in the benzene control group (i.e. 10 weeks as compared with 12½ weeks), while the total tumor yield was at least as great as in the control group.

Similarly, with 9,10-dimethyl-1,2-benzanthracene the average response was delayed from 13 weeks, in the case of 0.1 per cent in benzene, to 23 weeks, in the case of 0.1 per cent in lanolin, with a commensurate reduction in total tumor yield. Once again, using a 1.0 per cent solution in lanolin, the average response was brought down to 12 weeks, whereas with a 2.0 per cent solution, it was as early as 8½ weeks with all survivors acquiring tumors in 13 weeks! (Compare groups IV to VII, Table I).

When this carcinogen was tested in liquid paraffin, the results were essentially the same as in lanolin, the average response being 23 weeks with 0.1 per cent, but 13½ weeks when a 1.0 per cent solution was employed. (Compare groups VIII and IX, Table I).

## SUMMARY AND CONCLUSIONS

The striking diminution in the carcinogenic potency of methylcholanthrene, which occurs when lanolin is used as the diluent (1,2,3), has been

TABLE I: EFFECT OF CARCINOGENS IN VARIOUS SOLVENTS

Group	Carcinogen*	Concentrations, %	Solvent	Effective Total†	Tumor-bearing animals			First tumor, weeks	Tumors in 50% survivors, weeks	Tumors in 100% survivors, weeks
					Papilloma	Carcinoma	Total			
I	MC	0.3	benzene	16	9	5	14	7	12½	18
II	MC	0.3	lanolin	18	2	1	3	16	28	—
III	MC	3.0	“	17	6	7	13	6	10	15
IV	DMBA	0.1	benzene	25	10	13	23	6	13	24
V	DMBA	0.1	lanolin	19	9	4	13	13	23	31
VI	DMBA	1.0	“	24	9	10	19	4	12	18
VII	DMBA	2.0	“	18	8	7	15	4	8½	13
VIII	DMBA	0.1	liq. par.	16	8	4	12	12	23	37
IX	DMBA	1.0	“	23	8	12	20	7	13½	23

\* MC=20-methylcholanthrene

DMBA=9:10-dimethyl-1:2-benzanthracene

† Number of survivors at the time of the appearance of the first papilloma in the group.

Though the method used with methylcholanthrene was slightly different from that used by the previous workers (1,2), in that applications were made only twice weekly and continued beyond the 14th week, the results were essentially the same as theirs. Thus, the time taken for 50 per cent of survivors to develop tumors (hereafter referred to as

confirmed, and found also to operate in connection with another carcinogen, 9,10-dimethyl-1,2-benzanthracene. In both cases, however, the inhibitory effect was completely nullified when the concentration of the carcinogen was raised. Furthermore, in the lower concentrations, an analogous diminution in carcinogenic potency was obtained by sub-

stituting liquid paraffin for lanolin as diluent.

These results are incompatible with the idea that the inhibitory effect of lanolin constitutes a specific anticarcinogenic action. They are readily accounted for, however, on grounds of inadequate concentration:

A 0.3 per cent solution of methylcholanthrene in benzene probably represents something approaching a 2.0 per cent effective concentration in the tissues,<sup>1</sup> since the benzene evaporates, leaving the carcinogen dissolved in the minute amounts of sebum on the skin. In the case of a solution in lanolin, the concentration remains unaltered and this (i.e. 0.3 per cent in the case of methylcholanthrene) is probably a subthreshold concentration for effective carcinogenesis.

The present results show that provided the concentration of the carcinogen is high enough, lanolin serves as an exceptionally favorable medium for facilitating carcinogenesis. In view of this, its recommended use as a preventive measure against occupational cancer (6) would seem to be of doubtful value.

Finally conclusions regarding the mechanism of carcinogenesis, based on the belief that the inhibitory effect of lanolin is specific (3), must be deemed invalid in the absence of more substantial support.

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#### REFERENCES

1. SIMPSON, W. L., CARRUTHERS, C., and CRAMER, W. Loss of Carcinogenic Activity when Methylcholanthrene is Dissolved in Anhydrous Lanolin. *Cancer Research*, **5**:1-4. 1945.
2. SIMPSON, W. L., and CRAMER, W. Sebaceous Glands and Experimental Skin Carcinogenesis in Mice. *Cancer Research*, **3**:515-518. 1943.
3. SIMPSON, W. L., and CRAMER, W. Sensitization of Skin by Carcinogenically Inactive Methylcholanthrene to Subsequent Carcinogenesis. *Cancer Research*, **5**:5-10. 1945.
4. TWORT, C. C., and TWORT, J. M. Some Factors Influencing the Development of Experimental Oil and Tar Tumours in Mice. *Lancet*, **1**:1108-1111. 1929.
5. TWORT, C. C., and TWORT, J. M. Studien über Krebsentstehung. Krebsbildungsfähigkeit. *Ztschr. f. Krebsforsch.*, **32**:491-519. 1930.
6. TWORT, C. C., and TWORT, J. M. On the Prevention of Mineral Oil and Tar Dermatitis and Cancer. *Lancet*, **1**:286-287. 1934.