THE PROPHYLACTIC EFFECT OF A COLLOID MATERIAL ON THE SKINS OF MICE PAINTED WITH VARIOUS TYPES OF CARCINOGENIC AGENTS

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INTRODUCTION

The colloid material (S.D. 2), which contains as active constituents 30 % tetrachlorethylenum and 10 % oleum pini, was prepared and supplied by Mr J. F. Moseley of Altrincham for tests on the skin. It is claimed that this material had benefited considerably certain forms of dermatitis, including that produced by mineral oil. We were therefore interested to discover whether its effect on tumour production in mice would resemble our lanolin-olive oil ointment.

EXPERIMENTS WITH COLLOID MATERIAL

Although numerous experiments on the skins of mice carried out in this department during many years of research had shown that a considerable degree of protection was afforded by lanolin against oil dermatitis and cancer and under certain conditions against tar cancer (Twort & Twort, 1935), we had little evidence that it afforded any protection against individual carcinogenic hydrocarbons like benzpyrene for example. For this reason we initiated three groups of experiments utilizing as carcinogen in the first group 3·4. benzpyrene (0·2 % in chloroform), in the second a spindle grade shale oil (our oil 55), and in the third a carcinogenic gas tar (our tar 4). Experiments completed some years ago had determined the approximate potency of these agents, but for various reasons we have deemed it necessary to perform at least one control experiment in each group. Some of the mice in each group were treated with lanolin-olive oil ointment (2 parts lanolin and 1 part olive oil) in order to compare its effect with that of the colloid. A modification of the colloid, which we shall describe later, was used also in some experiments. In the experiments with benzpyrene and shale oil the carcinogenic agent was applied five times weekly in the morning, and the second agent was applied the same days in the afternoon some 6 hr. afterwards. In the experiments with the gas tar, however, the mice were painted with the tar on Mondays and Thursdays, and the second agent was applied on the remaining days of the week except Sundays. Relatively small numbers of mice were utilized in all of the experiments, and for confirmatory reasons the experiments with benzpyrene were repeated twice and those with shale oil either once or twice. Because the number of mice in each experiment was small we did not make use of our customary methods for comparing results (Twort & Twort, 1933) but utilized the number of tumours, the average time in weeks before each tumour appeared, and the addition cumulative frequency of tumours at definite periods, for this purpose. By the addition cumulative frequency of tumours we mean not the actual number of living animals which bear tumours at a particular time, but the estimated percentage number which would have borne tumours had all the mice survived to this period (Twort & Twort, 1933).

In the first group of experiments with benzpyrene there were three controls and a similar number in which the mice were treated with lanolin or colloid. Our results are given in Table 1, where abbreviations are as follows: C = control; L = lanolin-treated; S = colloid-treated; A = number of mice utilized for each experiment; LW = average life in weeks; T = total number of mice which bore tumours during the experiment; TW = average time in weeks for each tumour to appear; T 10 = number of mice which bore tumours at 10 weeks; CF 10 = addition cumulative frequency of tumours at 10 weeks; CF 100 = number of weeks elapsing before the CF reached 100 %; D = duration of experiment in weeks. Under CT, LT, and ST are given our results when all the controls, all the lanolin-treated, and all the colloid-treated, are considered as one experiment. All survivors were killed at 40 weeks:

Table 1

	A	LW	T	TW	T 10	CF 10	CF 100	D
C	10	21·8	8	10·7	4	50	·20	34
L	10	16·5	7	13·1	2	29	20	26
S	10	28·7	9	17·6	0	0	37	40
C	15	16·7	13	11·0	6	42	17	29
L	15	21·4	14	12·6	4	28	18	32
S	15	23·8	12	16·2	1	7	26	34
C	15	20·5	12	12·2	4	28	19	26
L	15	23·3	11	16·5	0	0	23	38
S	15	25·7	9	20·8	0	0	28	40
CT	40	19·7	33	11·2	14	40	20	34
LT	40	20·4	33	14·1	5	19	20	38
ST	40	26·1	30	18·2	1	2	37	40

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Examination of the data given in the table from left to right reveals that there was little average difference in the duration of life among the mice treated with lanolin and the controls, but quite a significant difference when we compare the colloid treated in this respect with the controls. Under T we may note that there was little difference in the actual numbers of tumours produced in the three types of experiments, but under TW we see that the average time for each tumour to appear in the control experiments was always less than in any of the experiments with lanolin. A much greater difference will, however, be noted if we compare the figures given for the colloid and the controls. Under T 10 it will be observed that the number of mice bearing tumours at 10 weeks was higher in all the control experiments than in those treated with lanolin, and at this time only a single mouse among all the colloid-treated animals bore a tumour. Data under CF 100 show that there was little significant difference in the corresponding experiments between the lanolin-treated and the control mice as regards the time elapsing before all the mice bore tumours. This indicates that although lanolin appears to delay tumours somewhat among the more susceptible animals it does not apparently retard tumour development in the more resistant ones. The colloid does not appear to act in this way, the 100 % period being reached in the three control experiments well in advance of the corresponding experiments with the colloid.

There appeared to be little difference in the average malignant lag of the tumours from the three types of experiments. Among the controls thirty of the tumours were diagnosed as malignant after an average of 17·0 weeks compared to twenty-two among the lanolin-treated after an average of 19·5 weeks and twenty-four among the colloid-treated mice after an average of 23·7 weeks. If we compare these figures with those given in the last three columns under TW we can see that the differences are approximately the same. Thus it appears that once the tumours have appeared neither the colloid nor the lanolin appreciably retards malignancy.

We know that when benzpyrene is applied daily to the skin of mice for some 6 weeks and the painting is then discontinued, subsequent daily applications of lanolin to the painted area does not appear to appreciably affect tumour incidence (Twort & Twort, 1939). We were interested to discover whether the colloid material would affect tumour incidence in this type of experiment. We therefore painted five times weekly, for 7 weeks, some seventy-five mice with an 0·2 % solution of benzpyrene in chloroform. At the end of this period the forty-six survivors were separated into two groups numbering twenty-three each, and there was at this time one tumour-bearing mouse in each group. One group received no further treatment of any sort, but the mice in the other group received applications of the colloid to the painted area five times weekly. Forty weeks after the first colloid application the survivors, numbering two in each group, were killed. Altogether six of the mice in the control bore tumours all of which arose before the fourteenth week, whereas twelve were noted among the colloid-treated mice, five of which did not appear until after the fourteenth week. Incidentally, although only two of the control mice bore tumours which were eventually diagnosed as malignant, there were nine among the colloid-treated which were diagnosed as such before death and all were confirmed with the microscope. Again, the tumours in three of the control mice were only transitory and disappeared before death, whereas among the colloid-treated all tumours persisted and continued to grow until death.

These experiments resemble in some respects those reported by Crabtree, who found that weak dilutions of certain organic chlor-compounds delayed considerably the advent of tumours in mice when applied on alternate days with a carcinogenic hydrocarbon, but increased the yield of tumours when used in place of the carcinogen after the latter had been applied for some weeks (Crabtree, 1940–1).

EXPERIMENTS WITH SHALE OIL

In the first series of experiments with shale oil some of the mice were treated with lanolin, some with the colloid, and the remainder were utilized as untreated controls. In the second series none of the mice was treated with lanolin, and in the third series there were no controls. As mentioned previously all the mice received applications of shale oil five times weekly, and the treatment with lanolin and the colloid was performed on the same days some 6 hr. afterwards. The results of these experiments are set out below in Table 2, where abbreviations are the same as those in Table 1. The numbers of mice bearing tumours after 20 weeks, T 20, and the addition cumulative frequency at this period, CF 20, are given, however, instead of the data at ten weeks as in Table 1:

Table 2

	A	LW	T	TW	T 20	CF 20	CF 100	D
C	15	6·8	2	10·5	2	100	13	19
L	15	17·1	2	24·0	1	20	29	30
S	15	11·5	3	14·0	3	100	19	23
C1	15	16·0	7	11·3	7	100	20	33
S1	15	17·6	7	16·4	6	69	28	40
L2	15	19·0	3	24·3	1	14	28	30
S2	15	16·8	5	15·2	4	53	24	40

Scanning the columns from left to right as in Table 1 we note under LW the short average life of the mice especially among the controls. We observe under T that the actual numbers of mice which developed tumours in the three types of experiments do not show appreciable differences. Under TW, however, we see that the average week before each tumour appeared in the various experiments differed considerably. In the first series there were only two survivors among the controls

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after 13 weeks and both animals bore tumours at this time. Among the colloid-treated all three survivors at 19 weeks bore tumours, whereas the first tumour did not appear in the lanolin-treated experiment until 20 weeks when there were five survivors. In the second series of experiments we note again that although seven mice eventually bore tumours in the control and colloid-treated the average time which elapsed before each tumour appeared was some 5 weeks earlier in the control. In the last series with lanolin and colloid we note that, as in the first series, the lanolin-treated mice developed their tumours on an average appreciably later than those treated with colloid. There is thus apparently some tumour delaying action on the part of the colloid in this type of experiment, but its effect is small compared with that of the lanolin.

EXPERIMENTS WITH GAS TAR

The last group of experiments consisted of a single series of four comprising twenty-five mice in each. As mentioned previously all the mice received applications of gas tar on Mondays and Thursdays. In the control experiment the mice received no additional treatment, but the others received applications of lanolin, colloid, and chloroform, respectively, on the other days of the week. It is well to point out at this stage that appreciable quantities of tar were utilized for each application (about 8 mg. per mouse). We knew from our previous experience with lanolin that the quantity of tar applied would affect our results, the lanolin affording some protection when applied alternately with relatively small quantities of tar or weak dilutions of it but having the reverse effect when relatively large quantities were utilized (Twort & Twort, 1935). Care was taken when applying the second agent that the material was well rubbed in and overlapped the area covered by the tar, a small camel-hair brush being utilized for the purpose. These experiments have now been in progress for 30 weeks, and they have been continued long enough for us to draw definite conclusions as regards the relative effect of the lanolin and colloid. Data as regards these experiments are set out in Table 3, where abbreviations are the same as those given in Table 2. The average life of the mice, however, is not given, but under SR is recorded the number surviving at 30 weeks in each experiment. Under CH are given our results with the chloroform-treated experiment:

Table 3

	A	SR	T	TW	T 20	CF 20	CF 100
C	25	8	12	19·4	5	33	26
L	25	10	15	19·3	10	64	24
S	25	8	8	24·1	1	8	—
CH	25	8	11	18·1	10	88	23

Reading the columns from left to right in the table we note that there is little difference in the number of survivors at 30 weeks but a considerable difference in the number of tumour-bearing mice at this time, fifteen among the lanolin-treated, and only eight in those treated with the colloid, compared to twelve among the controls. Under TW it will be noted that the average time in weeks for each tumour to appear in the colloid-treated experiment was nearly 5 weeks later than in the controls, and actually one mouse is still tumourless in the former experiment. We will notice under T 20 and CF 20 that the actual number of mice which developed tumours and the addition cumulative frequency of tumours at 20 weeks show significant differences in the four experiments. Only one mouse had at this time developed a tumour in the colloid-treated experiment compared to five in the control and ten each in the others. Under CF 100 it will be noted that the 100 % tumour period was reached somewhat earlier in both the lanolin- and chloroform-treated experiment than in the control but appreciably later in the colloid experiment, one mouse still being tumourless at the present time, 30 weeks after the experiment was started. These experiments thus indicate that the colloid delays the advent of tumours in this type of experiment and that the chloroform and lanolin hasten somewhat their arrival.

EXPERIMENTS WITH A MODIFICATION OF THE COLLOID

As the experiments with shale oil revealed that the colloid as constituted apparently offered much less protection than the lanolin against the production of tumours produced by this type of carcinogen a modified form of the colloid in which 25 % of lanolin was incorporated was tested on the skin. Three experiments with this modified colloid were initiated, utilizing as carcinogen, benzpyrene, shale oil, and gas tar respectively. The results of these experiments showed fairly conclusively that this modified colloid resembled very closely the lanolin in its effect when used in conjunction with benzpyrene or gas tar but gave little or no more protection than the original colloid when used with shale oil.

DISCUSSION

Our experiments indicate that the colloid material has some definite delaying action as regards tumour production when applied to the skin of mice alternatively with gas tar, 3·4. benzpyrene, or shale oil. It appears, however, to increase the yield of tumours when applied after the skin has been rendered hyperplastic by the applications of benzpyrene. Results of experiments performed in this department some years ago indicated that skins rendered hyperplastic by the applications of benzpyrene were not significantly affected as regards subsequent tumour formation by treatment with lanolin. Previous experiments have shown also that lanolin affords some protection against tar tumours only when relatively small quantities or weak dilutions of tar are applied (Twort & Twort, 1935). Our experiments indicate that appreciable protection is afforded by the colloid even when relatively large quantities of tar are used for each application. The colloid is, however, apparently much less efficacious than lanolin in delaying the advent of tumours produced by shale oil.

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It would appear on the evidence of the experimental results of Crabtree with organic chlor-compounds that the prophylactic effect of the colloid was probably mainly due to the presence of tetrachlorethylenum. Our experimental results with a mixture of this compound and lanolin in the modified colloid gave strong indications that the inhibitory effect of the lanolin and tetrachlorethylenum were considerably diminished when acting together. In the experiments with shale oil the tendency of the skins to remain for some time relatively smooth and free from ulceration when treated with lanolin was noted in contrast to those treated with the colloid. Ulceration of the skin of mice treated with the latter material was almost as frequent as in the controls.

CONCLUSIONS

A colloidal material (SD 2) delayed the advent of tumours when used in conjunction with benzpyrene, gas tar, or shale oil.

The protective action of this colloid exceeded that of lanolin when used alternatively with either benzpyrene or gas tar, but it afforded less protection than lanolin when used in conjunction with shale oil.

The incorporation of some 25 % of lanolin in the colloid rendered it less effective than the original except possibly when used in conjunction with shale oil.

Skins rendered hyperplastic by the applications of benzpyrene gave a higher yield of tumours when subsequently treated with the colloid than when left untreated.

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