THE UTILITY OF LANOLIN AS A PROTECTIVE MEASURE AGAINST MINERAL-OIL AND TAR DERMATITIS AND CANCER

By C. C. TWORT and J. M. TWORT
(*Manchester Committee on Cancer*)

Cancer Research Department, Victoria University of Manchester

When mineral oils and tars come into contact with the skin of susceptible individuals among certain species of animals (man, mice, rabbits, etc.), they may induce a variety of inflammatory conditions. These conditions may be conveniently divided into: dermatitis which is an affection of epidermis and dermis, but is essentially a disease of the latter; and cancer which is again an affection of epidermis and dermis, but is essentially a disease of the former. While dermatitis may arise soon after contact with tar or oil, cancer, on the other hand, almost invariably requires contact over a long period before it becomes manifest. It is important to bear in mind that dermatitis is not necessarily a precursor of cancer.

Information gained from laboratory experiments led us to advocate, during recent years, the utilisation of lanolin for the prevention of mineral-oil dermatitis and cancer. The striking benefit derived from this method of preventive treatment by mineral-oil workers resulted in employers, whose workmen come into contact with tars and their products, seeking our advice as to whether the same treatment would not be equally efficacious in preventing similar troubles caused by tar. We were, unfortunately, until recently not in a position to advise the utilisation of lanolin in this respect, but during the last two years we have studied this question more closely, and our object is now to place our results on record so that those interested may be fully cognisant of our present views. A note upon this subject has already been published (*Lancet*, 1934, i, 286) and we propose here to give some of the experimental details promised in our previous communication.

The carcinogenicity of our agents, measured by the response of the mouse, is given in numerical terms as the carcinogenic potency (P.) of the agent. When comparing one agent with another we also, for convenience, give the relative carcinogenic potency (R.P.), one of the agents always being given an R.P. of 100, so that it is easy to see the percentage variation of the second. We cannot discuss here our methods of arriving at the P. of an agent. They have been described in some detail elsewhere (*J. Hygiene*, 1930, **29**, 373; *J. Industr. Hygiene*, 1931, **13**, 204; *Amer. J. of Cancer*, 1933, **17**, 293) and it will suffice for our present purpose if we state that they are based upon the yield of tumours observed as a result of the applications of the agent compared with the yield in a similar period of time given by what we call our hypothetical standard agent (H.S.A.).

Most of the experiments fall into one or other of two main groups, viz. (1) bi-weekly experiments wherein the carcinogenic agent is applied over the scapular region of 100 mice twice a week, the prophylactic agent, if used, being applied on four of the intervening days; and (2) daily experiments wherein the carcinogenic agent is applied five times per week, usually in the early morning, the prophylactic agent, if used, being applied also five times per week, usually in the late afternoon. In the latter category of experiments the interval between carcinogenic and prophylactic agent applications is in most cases six hours.

Before we enter into a discussion of our experiments it may be well to say a few words concerning the chemical and physical constitution of the agents we have utilised, some of the most important features to take into account being:

(1) The concentration of the cancer-producing principle. In many petroleum oils obtained from wells this is relatively low, in shale oils it is relatively high, while in some gas tars it may be relatively very high, although not so high as in most synthetic tars.

(2) The concentration of inflammation-producing principle. In refined petroleum oils this is low, being somewhat higher in refined shale oils. It appears to be high in most unrefined or what are called unfinished mineral oils, and is usually very high in gas tars, probably even if not actively carcinogenic.

(3) The concentration of cell toxins in the agent. The type of substances we have in mind are the phenols, etc. Such substances do not for practical purposes appear to occur in mineral oils, but, on the other hand, are abundant in gas tars, and especially in creosotes.

(4) The concentration of the epilation principle. When considering mineral lubricating oils and gas tars separately the amount of epilation principle in the agent seems, as a rule, to vary directly with the amount of the carcinogenic principle, but if a mineral oil and a gas tar of apparently equal carcinogenicity are compared it will be found that the former is a more active epilator than the latter.

(5) The concentration of the excoriation principle. We have in mind here the substances which lead to drying and cracking of the surface epithelium, followed by scabbing and definite ulceration. Although some of the substances referred to may be the primary cause of the ulceration, the latter is presumably often the direct result of damage inflicted to the epithelium by the animal itself in an endeavour to remove mechanically with the paw the agent applied. Among agents of apparently equal carcinogenicity, the excoriation principle is more abundant in mineral oils than in gas tars. It is not improbable that mineral oils irritate the animal more than do gas tars, and it may be that the

phenols present in the latter are partly responsible for this difference. The excoriation principle may not exist as a single substance but may really be a combination of the substances previously mentioned, which results in this special manifestation of damaged epidermis.

(6) The concentration in oils of substances having a laxative effect and inhibiting assimilation of foodstuffs. These are presumably more abundant in mineral oils than in gas tars. Their importance in relation to the general health of the animal, and consequently on the tumour yield, will be seen later. In addition to a consideration of the varying amounts of the different chemical constituents making up our agents, we have to bear in mind several further points.

(7) The ease with which the agent is able to penetrate the surface layers of the epidermis. Mineral oils appear to penetrate easier than gas tars.

(8) The aid to penetration which may be offered by an animal fat. From (7) we must assume that tars would receive more aid than mineral oils.

(9) The differences in saponifiability of, for instance, olive oil, lanolin and mineral oils. It is much more difficult to remove lanolin from the skin by the aid of soap and water than it is to remove olive oil, while it is still more difficult to remove mineral oils.

(10) The undoubted aid offered by an animal fat to the mechanical removal of hydrocarbons by soap and water.

(11) In the case of dusty occupations, such as when pitch is utilised for the making of briquettes, one has to consider the possibility of an animal fat aiding the pitch to adhere more closely than otherwise to the surface of the skin.

(12) The extreme delicacy of the epidermis of the mouse, compared with that of man, has to be taken into account when surmising, from animal experiments, what is likely to happen in man—a very important point.

The quantity of the agent applied

A knowledge of the quantity of the agent applied is essential, the yield of tumours being, within limits, proportional to dosage of agent. It is sometimes difficult to apply very small quantities of a substance directly, so that in order to vary the dose of the agent we customarily dilute with a volatile solvent, the animal being eventually in contact with a small but definite quantity of the pure substance. For exact experiments we dilute with chloroform, or more rarely with benzene or alcohol, any such diluents being much more satisfactory for measuring the dosage than, for instance, liquid paraffin. Although inert carcinogenically the latter has the disadvantage of protecting somewhat the skin from the noxious action of a carcinogenic agent, while chloroform, benzene, etc., although they may delay tumour formation, have at least the merit, by virtue of their volatility, of eventually leaving a pure residue of tar or oil to act upon the animal.

When painting with pure agents there is a tendency for the painter to apply an amount more or less indirectly proportional to the toxicity of the agent for the animal, in order that too high a death rate should not supervene. Thus there is, as a rule, a tendency for less gas tar to be applied than shale oil, and less shale oil than some of the other mineral oils. There is no such error when the agents are diluted with a volatile solvent, an approximately even amount being applied in all cases, with consequently comparative results of greater value. Needless to say evaporation from the stock solutions must be very carefully guarded against. A few examples of "daily" dilution experiments with oils and tars are given in Tables I and II respectively.

Table I. *"Daily" experiments. The effect of dilution of oils with volatile solvent*

Oil no.		Carcinogenic potency (P.)	Relative carcinogenic potency (R.P.)
23	Pure	42	100
	10 % in chloroform	4	10
68	Pure	53	100
	10 % in chloroform	5	9
69	Pure	249	100
	10 % in chloroform	19	8
55	Pure	244	100
	10 % in chloroform	27	11

Table II. *"Daily" experiments. The effect of dilution of tars with volatile solvent*

Tar			P.	R.P.
Gas tar 2	10 %	in chloroform	296	100
	5 %	,,	212	72
	1 %	,,	112	38
Synthetic tar B/19	1 %	,,	1339	100
	0·5 %	,,	926	69
	0·1 %	,,	122	9
	0·05 %	,,	5	0·4
	0·02 %	,,	+	+
	0·01 %	,,	0	0

Besides "dilution" experiments we have performed others wherein the amount of pure tar applied is small, the tar being spread on a glazed porcelain plate from which it is transferred by means of a clean brush to the animal. Experiments performed by this technique are designated "film" experiments in this paper, and we are of opinion that, as regards quantity of tar, conditions governing this type of experiment approximate closest to those prevailing among most tar workers. It will be seen by consulting Table III that there is here, as in the dilution experiments, a profound reduction in the tumour yield; in other words, the quantity of the agent applied has a marked influence on the resulting carcinogenic potency obtained. Mice did not tolerate well daily applications of pure gas tars in quantity, so that controls of this nature were not performed. Another way of varying the amount of tar applied during the course of an experiment is, of course, by adjusting the frequency of the applications. As stated previously we usually apply our agents five times or twice a week, but we have on some occasions made the applications only once or twice a fortnight. In this case, even although the agent may be very powerful, the tumour yield falls off perceptibly. Such an experiment is shown in Table IV.

Table III. *Film experiments*

	Agent		P.	R.P.
D.	Gas tar 2, pure	Control	—	—
		Film	47	—
B.	Gas tar 2, pure	Control	109	100
		Film	11	10
	Gas tar 2, and 10 % lanolin	Control	214	100
		Film	*52*	*24*
	Gas tar 2, less phenols and carbon	Control	168	100
		Film	—	—

D. Indicates that applications were made five times per week.
B. Indicates that applications were made twice per week.

Table IV. *Applications at long intervals*

Agent	Applications per fortnight	P.	R.P.
Synthetic tar B/3	4	2714	100
	2	490	18
	1	262	9

The avoidance of mineral oil cancer and dermatitis

Our direct experiments with carcinogenic and protective agents can be divided into four main groups, viz.:

(*a*) Bi-weekly applications of the carcinogenic agent, with applications of the protective agent on the other days of the week except Sundays; the interval between the applications of the two separate agents being not less than 24 hours.

(*b*) Daily applications of the carcinogenic agent and the protective agent except on Saturdays and Sundays, the interval between the applications of the two separate agents being as a rule six hours.

(*c*), (*d*) Applications made twice and five times per week as in (*a*) and (*b*), the carcinogenic and protective agent being blended instead of being applied separately.

Each of the four groups is further subdivided into those relating to mineral oils and those relating to tars.

(*a*) *Bi-weekly applications*

We will first discuss experiments involving bi-weekly applications of mineral oil. In some of these experiments the animals were also painted on the remaining four days of the week (Sunday being excluded) with a second agent which aimed at having a protective influence on the noxious action of the mineral oil. A series of results are given in Table V.

It will be noted that there was some protection in all instances except in the case of wool fats A and D, both of which appeared to act in the opposite direction. As the lanolin and wool fats were diluted with olive oil (which,

https://doi.org/10.1017/S0022172400019045 Published online by Cambridge University Press

as we shall see in a moment, when acting alone has a definite protective action), it is somewhat difficult to evaluate the relative effects of the other ingredients. Lanolin would seem to be the best, but in the oil 8(2) experiments wherein only a single benign tumour was obtained when the animals were treated with lanolin, the amount of mineral oil applied was by mistake relatively very small.

Table V. *Bi-weekly applications*

Carcinogenic agent		Treated with	P.	R.P.
Shale oil	8 (2)	—	85	100
	8 (2)	Lanolin	+	+
	55	—	40	100
	55	Lanolin	15	38
	55	Wool fat A	68	170
	55	Wool fat B	11	28
	55	Wool fat C	36	90
	55	Wool fat D	54	135
	55	Skin soap R	24	60
	55	—	54	100
	55	Lanolin (Saturday)	23	43

(*b*) *Daily applications*

As already remarked, when the carcinogenic agent is applied five times per week instead of twice, it is in all experiments, unless otherwise stated, applied in the morning, the prophylactic agent, if used, being applied in the afternoon, about 6 hours later. For mineral oil experiments daily applications are much superior to bi-weekly applications. Some of our results are given in Table VI.

Table VI. *Daily applications*

Carcinogenic agent		Treated with	P.	R.P.
Shale oil	8 (2)	—	293	100
	8 (2)	Lanolin	16	5
	8 (2)	—	258	100
	8 (2)	Soap and water after 6 hours	362	140
	8 (2)	Soap and water after 1 hour	72	28
	8 (2)	Soap and water immediately	0	0
	8 (2)	Petroleum ether	301	117
	8 (2)	Petroleum ether (morning)	219	85
	55	—	244	100
	55	Olive oil	53	22
	55	Glycerine	204	84
Petroleum oil	69	—	585	100
	69	Lanolin	23	4

The task of the prophylactic agent is here apparently not so severe as it was in the previous group of experiments, the lanolin offering a remarkable degree of protection to the noxious action of the mineral oils. Olive oil appears to be much more efficacious than glycerine. Soap and water seems to be only of utility if the shale oil is mechanically removed by washing almost immediately after coming in contact with the skin surface (see later for comparative reaction of man and mouse). Delayed washing may possibly do more harm than good, the removal of any of the harmful shale oil being seemingly neutralised by removal of some of the protective natural skin greases. Further experiments in this direction are needed.

efficacious in protecting the animal from mineral-oil cancer, it appeared to increase the yield of tar tumours. The part played by the olive oil and the neatsfoot oil needed investigation, and it was for this reason, combined with the fact that the death rate in Group 1 was high, that the Group 2 experiments were performed. Although they have not yet reached completion we have given the potency readings found up to date, and it will be seen that they in the main confirm our Group 1 findings. The increase in the yield of tumours among animals treated with pure neatsfoot oil is interesting, and it is unfortunate that we had not at the time animals available for a parallel olive-oil experiment.

Table IX. *Bi-weekly applications of carcinogenic agent*

Gas tar no.	Group 1	P.	R.P.
2	Control	109	100
	Treated lanolin-olive oil	194	178
3	Control	131	100
	Treated lanolin-neatsfoot oil	354	270
4	Control	243	100
	Treated lanolin-neatsfoot oil	298	123
5	Control	70	100
	Treated lanolin-neatsfoot oil	159	227
	Group 2		
3	Control B	138	—
4	Control B	204	100
	Treated lanolin-olive oil	340	167
	Treated lanolin-neatsfoot oil	396	194
	Treated neatsfoot oil	456	224
5	Control B	51	—

It might be concluded from the results of these experiments that the use of lanolin by workmen exposed to contact with gas tars was a dangerous procedure, likely to increase instead of decrease the number of cases reported in subsequent years. However, as stated elsewhere, one must be wary of applying animal figures directly to man, and if our results with dilutions of gas tar, film experiments, etc., are examined, it will be seen that they tend more to approximate those obtained with mineral oils. The results of the experiments referred to will be found in Table X.

Table X. *Bi-weekly applications of carcinogenic agents*

Agent		P.	R.P.
Gas tar 2, film	Control	11	100
	Treated lanolin-olive oil	4	36
Gas tar 2, 5 % in $CHCl_3$	Control	18	100
	Treated lanolin-olive oil	1	6
Creosote, crude	Control	15	100
	Treated lanolin-olive oil	2	13
Synthetic tar B/3	Control	2714	100
	Treated lanolin-olive oil	1120	41

Here we have definite evidence that the lanolin-olive oil ointment is protecting the animal, the difference between these experiments and those given in Table IX being presumably due entirely to the difference in the dosage of the tar applications, the creosote in this case acting as a dilute tar. The difference is certainly not due entirely to the degree of carcinogenic activity of the agent, witness the decrease in tumour yield by more than a half in the case of the highly carcinogenic synthetic tar.

Some further animals subjected to bi-weekly applications of pure and dilute gas tars were later treated with several other substances in an endeavour to find a more efficient remedy than lanolin. The results of some of these experiments will be found in Table XI.

Table XI. *Bi-weekly applications of carcinogenic agents*

Carcinogenic agent	Protective agent	P.	R.P.
Gas tar 2, pure	Control	109	100
	Glycerine	74	68
	Skin soap C	70	64
	Chloroform	38	35
	Liquid paraffin	51	47
	D.N.B.C.	19	18
Gas tar 2, film	Control	11	100
	Skin soap R	14	127
Gas tar 2, 5 % in $CHCl_3$	Control	18	100
	Olive oil	5	28
	Wool fat F	5	28
	Wool fat P	59	328
	Wool fat S	29	161
	Wool fat T	9	50

D.N.B.C. 1 % of 2-4-dinitrobenzylchloride in liquid paraffin.

In this group of experiments we see that, when using crude gas tar in the ordinary manner, the five protective agents tested all reduced the action of the tar, whereas we have just seen that lanolin acted in an opposite direction. The dinitrobenzylchloride seemed to give the best protection, and we remember that this substance appeared to be beneficial when added to mineral oils. The only other thing of note is that, while olive oil and wool fat F decreased the tumour yield, some of the other wool fats markedly increased it. We must mention that all the wool fats were made into a paste with olive oil, but some required more oil than others, wool fat F requiring the most on account of its granular nature.

A comparison of this table with Table V (p. 135) shows one difference between the efficacy of the several protective agents utilised towards gas-tar and oil cancer. The differences as regards dermatitis are not recorded in the tables, but it may be stated that in a general sense dermatitis and carcinogenesis run more or less parallel, and an agent which protects from the carcinogenic constituents of a tar or oil also diminishes the amount of dermatitis. As a matter of fact the amount of epilation is often a fairly good guide as to the progress of an experiment, but in this case, as we have seen, oils must not be compared with tars.

(*c*), (*d*) *Blending of mineral and saponifiable oils*

Another method adopted in an endeavour to neutralise the carcinogenic effect of mineral oils was by the addition to them of a second agent. To this end several mineral oils were blended with various saponifiable oils, etc., and a certain measure of protection to the animal was obtained. The degree of protection varied greatly, both as regards the type of mineral oil and the type of protective agent, but there is often a good deal of difficulty in gauging accurately the results of this category of experiment owing to the small number of tumours which may be available. In Table VII are given the results of some "daily" experiments. A glance at this table shows clearly that the pure lanolin is much superior to the various wool fats prepared from it. Sperm oil also considerably lowers the activity of shale oil, but not so much as does lanolin. The animals of the beeswax and oleic acid experiments lived very badly, so that our results here are unreliable, although it is highly probable that the presence of these substances appreciably lessens the activity of the carcinogenic agent. Stearic acid also appeared to act in the same direction. Among other agents we found that 2–4-dinitrobenzylchloride appreciably delayed the advent of tumours when 1 per cent. was added to mineral oils (see

Table VII. *Blends of mineral oils and saponifiable oils, etc.*

Agent		P.	R.P.
Shale oil 8 (2)	Control	293	100
	and 10 % lanolin	31	11
	and 5 % sperm	92	31
	and 10 % sperm	87	30
	and 20 % sperm	68	23
Shale oil 55	Control	244	100
	and 10 % wool fat A	100	41
	and 10 % wool fat B	208	85
	and 10 % wool fat C	269	110
	and 10 % wool fat D	214	88
	and 10 % ionised olive oil	62	25
	and 10 % ionised castor oil	142	58
	and 5 % oleic acid	112	46
	and 1 % stearic acid	118	48
	and 1 % beeswax	74	30
	and 2·5 % beeswax	122	50

N.B. Applications five times per week in all experiments.

Table VIII. *Daily experiments with ortho- and* 2–4-*dinitrobenzylchloride*

Agent		P.	R.P.
Shale oil 55	Control	244	100
	and 1 % Ortho-D.N.B.C.	152	62
	and 1 % 2-4-D.N.B.C.	90	37
Pennsylvanian II	Control	2	100
	and 1 % Ortho-D.N.B.C.	3	150
	and 1 % 2-4-D.N.B.C.	0	0
Borneo oil 49	Control	64	100
	and 1 % 2–4-D.N.B.C.	0	0
Californian oil 50	Control	86	100
	and 1 % 2–4-D.N.B.C.	22	26

N.B. This was rather a poor group of experiments owing to the detrimental effect of the dinitrobenzylchloride (D.N.B.C.) on the general health of the animals.

Table VIII), but meanwhile the results of these and similar experiments must be considered only of academic interest.

In conclusion it may be stated that of all the protective agents tested by us anhydrous lanolin appears to be the most efficacious, at least among saponifiable oils. We have found that, as a rule, the latter give a greater relative degree of protection to the noxious influence of the more strongly carcinogenic mineral oils than they do to the weaker ones, and that they also give a greater relative protection when the mineral oil is clean and "bright" than if it is an unfinished, dirty oil. We shall enlarge upon these points when considering the part played by cancer-inhibiting substances present in different mineral oils and tars.

Protection from tar cancer and dermatitis

So far as we are aware, all workers who have carried out investigations related to this subject arrived at the conclusion that an excess of fat on the surface of the skin is conducive to an increase in tumour yield over and above the yield ordinarily obtained from gas tar applications. The excess fat may be brought about either by adding it to the tar (applying the fat shortly before painting with tar being in effect practically the same as direct addition), or by feeding the animals with a large amount of fat during the course of the experiment (Watson, A. F. and Mellanby, E., *Brit. J. Exper. Pathol.* 1930, **11**, 311). Similar results are obtained if gas pitch is used instead of tar, and it is generally assumed that the explanation of this increased yield of tumours is that the animal fat allows the tar or pitch to penetrate more easily than is the case with a dry skin.

A. *Bi-weekly experiments*

The benefit derived from the protective measures adopted against mineral-oil dermatitis and cancer prompted us to examine the question of tar dermatitis and cancer, and although we are not meanwhile in a position to discuss all the experiments we have undertaken in this direction, we have sufficient data available to draw certain provisional conclusions. The first group of experiments we shall consider is that in which the tar was applied bi-weekly, and as in the case of the similar experiments with mineral oils, the protective agent, when used, was applied on the remaining four days of the week (Sundays excluded). The tar and mineral-oil applications were habitually made on Wednesdays and Saturdays, but in view of the fact that by this procedure the carcinogenic agent was allowed a free forty-eight hours of contact without a lanolin application over the week-end, the tar or mineral-oil application was later sometimes shifted to Tuesday and Friday. The results of some experiments with four gas tars and lanolin ointment are shown in Table IX.

These experiments confirm those of a somewhat similar type performed by Watson, Mellanby and Leitch, and show that while we found lanolin so

B. *Daily experiments*

Application of the carcinogenic agent five times instead of twice a week gives us further information. As we have already remarked, experiments of this nature, performed with pure gas tar in the ordinary manner, were useless; we thus have to rely upon dilutions, etc. While it is customary for the applications of the carcinogenic agents to be made from 7 to 9 a.m., the protective agent to be tested being applied about six hours later, we have in some instances reversed the procedure, the protective agent being applied in the early morning and the carcinogenic agent in the afternoon. In the former case we may consider that the carcinogenic agent has eighteen and the protective agent six hours in which to exert its action, while in the latter case the conditions are reversed. We should expect the latter conditions to be more favourable, but our results only give differences within those of experimental error. It will be noted that, in several of the tables, two apparently exactly similar control experiments give widely different results. This is because the technique varies in different groups of experiments, each variable control being given its relative potency figures of 100, with the appropriate figures allotted to the comparative experiments belonging to the particular group. A study of the figures in Table XII leaves one in no doubt as to the efficiency of the lanolin-olive oil mixture in delaying the advent of tumours resulting from the action of dilute gas tars, the figures being as, or even more striking than was the case with mineral oils. If one refers to Table XIII it will be seen that most of the other substances tried offered some measure of protection to the animal, but it is small compared with the protection obtained from the lanolin. It is probable, however, that the potency found for control B was lower than it should have been, which would result in the protection obtained with these substances being apparently rather smaller than it should have been.

Table XII. *Daily applications of carcinogenic agents*

Carcinogenic agent	Protective agent	P.	R.P.
Gas tar 2, 5 % in $CHCl_3$	Control A	212	100
	Lanolin-olive oil (p.m.)	7	12
	Lanolin-olive oil (a.m.)	2·5	4
Gas tar 2, 1 % in $CHCl_3$	Control	112	100
	Lanolin-olive oil	5	5
Synthetic tar B/19, 1 % in $CHCl_3$	Control	1339	100
	Lanolin-olive oil	400	30

Table XIII. *Daily applications of carcinogenic agent*

Carcinogenic agent	Protective agent	P.	R.P.
Gas tar 2, 5 % in $CHCl_3$	Control B	60	100
	Glycerine	38	63
	Chloroform	20	33
	Skin soap R (p.m.)	35	58
	Skin soap R (a.m.)	50	83
	Oleic acid	51	85

C, D. *Blends of tar and saponifiable oils*

A series of experiments more or less parallel with those performed with oils was carried out with tars, the protective agent to be tested being blended with the tar instead of being applied separately. The results of some of the lanolin experiments are shown in Table XIV, and by comparing them with those given in previous tables many interesting points of difference will be noted.

Table XIV

Agent		P.	R.P.
B. Gas tar 2, pure	Control	109	100
	and 10 % lanolin	214	196
	and 40 % lanolin	83	76
	and 70 % lanolin	21	19
	and 90 % lanolin	0	0
Gas tar 2, film	Control	11	100
	and 10 % lanolin	52	473
D. Gas tar 2, 5 % in $CHCl_3$	Control B	60	100
	and 0·5 % lanolin	131	218
Gas tar 2, 1 % in $CHCl_3$	Control	112	100
	and 10 % lanolin	143	128
	and 50 % lanolin	69	62
Synthetic tar B/19, 1 % in $CHCl_3$	Control	1339	100
	and 0·1 % lanolin	757	57

B. Bi-weekly applications. D. Daily applications.

In the first place the addition of as much as 10 per cent. of lanolin to gas tar, whether crude or as a 5 or 1 per cent. dilution in chloroform, markedly raised the carcinogenic potency, while separate treatment with lanolin of animals painted with these same three tars, although it increased the potency of the crude tar, profoundly lowered that of the two tar dilutions. With the addition of 40 per cent. lanolin the potency of the blend fell below that of the pure gas tar, while with an addition of 90 per cent. lanolin no tumours at all were obtained. Lanolin, whether blended with or applied separately to the highly carcinogenic synthetic tar, perceptibly delayed tumour formation.

Blends of tars with some other materials were next undertaken, the results of these experiments being shown in Table XV.

Table XV

Agent		P.	R.P.
B. Gas tar 2, pure	Control	109	100
	and 10 % petroleum grease	22	20
	and 10 % olive oil	18	17
	and 10 % liquid paraffin	31	28
	and 10 % D.N.B.C. sol.	28	36
D. Gas tar 2, 5 % in $CHCl_3$	Control C	94	100
	and 0·5 % wool fat F	125	133
Synthetic tar B/19,	Control A	210	—
1 % in liquid paraffin	Control B	261	100
	1 % in oleic acid	65	25

D.N.B.C. A 1 % dilution in liquid paraffin.
B. Bi-weekly applications.
D. Daily applications.

It will be observed that all four materials tested when added to pure gas tar, and applied twice a week, caused a considerable diminution in the carcinogenic potency. The petroleum grease utilised was of a consistency more or less similar to that of pure anhydrous lanolin, but the potency of the grease-tar blend was little more than one-tenth of that given by the parallel lanolin-tar blend. Olive oil gave splendid protection, and it is important to remember in this connection that while this oil is mixed with the lanolin when the latter is applied separately, it is the pure anhydrous lanolin which is used in making up all lanolin blends.

In an attempt to gain some knowledge of the mechanism of the action of the lanolin, and incidentally of that of the tar as well, we have instituted experiments on some of the constituents of both the tar and the lanolin. Although we have not advanced far in this direction, it may be worth while giving the results obtained to date. Our co-worker Dr Bottomley prepared phenol- and carbon-free tar by repeatedly washing our crude gas tar no. 2 with alkali. The product was divided into three portions, the first serving as the control, and the second and third having in the one case the phenols and in the other the carbon re-added in the proportion equivalent to that in the original tar. Our co-worker Mr Lyth was meanwhile making a separation of lanolin, but as a sufficient amount of the products obtained by him were not available, we utilised the commercial products referred to in the tables as wool fats. We are indebted to Dr Speakman of Leeds and Messrs Croda, Ltd., of Goole, for having kindly supplied us with these materials.

A number of experiments with the commercial wool fats have already been mentioned. In general we surmise from our results that the wool fats are not so efficacious as the whole lanolin in preventing mineral-oil dermatitis and cancer. Our results with tars are not so easily summarised, and the complication in many of the experiments of the olive oil, of necessity added to the lanolin and wool fat in order to have a workable paste, renders the issue very confused. A few experiments, to be mentioned shortly, have been recently undertaken in an endeavour to overcome this complication.

The results of some experiments on the tar itself and also with gas pitch are given in Table XVI. Firstly we notice that the pitch had a carcinogenic activity below that of the gas tar, but that the tarry extract of the former obtained by means of alcohol had a considerable increase in activity. The residue, consisting mostly of carbon, was almost inert, sufficient tar being retained to induce a few delayed tumours. It may be recalled in passing that early pitch tumours of mice have a great tendency to disappear spontaneously; and we have wondered whether this fact has any connection with the lower percentage death-rate among notified cases of pitch cancer as compared with the death-rate among notified cases of gas-tar workers' and mule spinners' cancers. Oil bitumen in two experiments was absolutely inert carcinogenically. As regards our tar experiments it will be seen that when the tar is deprived of both phenols and carbon, the carcinogenic potency is increased (? partly due to removal of non-carcinogenic diluents). Not only is this so when the tar is applied in the ordinary manner but also when it is applied as a film or diluted in a volatile solvent. On the contrary, when lanolin treatment is instituted, or lanolin is blended with the tar, the absence of the phenols and carbon appeared to lower the tumour yield. At the moment we are at a loss to account for the fact that, while crude tar deprived of both phenols and carbon induced an increased yield of tumours, deprived of either one of them it apparently induced less. Unfortunately the death-rate among this group of animals was unusually high, and it may be that subsequent, more reliable, experiments will enable us to evaluate better the various parts played by the different constituents of our tars and fats in the production and prevention of cancer.

Table XVI. *The effect of carbon and phenols*

	Agent		P.	R.P.
B.	Gas pitch	Crude	44	100
		Extract (less carbon)	186	423
		Residue (mostly carbon)	4	9
	Oil bitumen	—	0	—
	Gas tar 2	Crude	109	100
		Less phenols	69	63
		Less carbon	28	26
		Less phenols and carbon	168	154
	Gas tar 2, film	Crude	11	100
		Less phenols and carbon	21	191
	Gas tar 2, treated lanolin	Crude	194	100
		Less phenols and carbon	39	20
	Gas tar 2, and 10 % lanolin	Crude	214	100
		Less phenols and carbon	157	73
D.	Gas tar 2, 10 % in $CHCl_3$	Crude	83	100
		Less phenols and carbon	124	149

B. Bi-weekly applications. D. Daily applications.

As olive oil in all experiments, whether with tars or oils, and whether utilised separately or blended, delayed tumour formation, it was thought advisable to dispense with this material for the making up of lanolin ointments, etc., so as to eliminate at least one complication. To this end several pastes were made up with chloroform in place of the oil, the animal results being given in Table XVII.

Table XVII

	Agent		P.	R.P.
B.	Gas tar 2	Control	109	100
		Treated lanolin—$CHCl_3$	74	68
		Treated beeswax—$CHCl_3$	52	48
D.	Gas tar 2, 10 % in $CHCl_3$	Control	83	100
		Treated lanolin—$CHCl_3$	19	23
		Treated beeswax—$CHCl_3$	31	37
		Treated wool fat F—$CHCl_3$	21	25

B. Bi-weekly applications. D. Daily applications.

The degree of protection offered by the three substances with which we are here concerned did not appear to be very dissimilar, but in view of the fact, as we have already shown, that chloroform itself seems to delay the

https://doi.org/10.1017/S0022172400019045 Published online by Cambridge University Press

advent of tumours, no very satisfactory conclusions can be drawn from this group of experiments. Moreover, it is by no means clear why chloroform should act in this manner, especially when one considers certain experiments of a similar nature to the above which have been performed with other volatile solvents.

Considerations as regards the human skin compared with the mouse skin

While we are of opinion that the reaction of the animal to our agents gives us valuable information as to the probable reaction of man to similar agents, we do not assume that man and animals will react in an exactly similar manner. Reasonable deductions from our animal experiments will only be obtained by a considered survey of the differences appertaining both locally and generally to the particular species of animal with which we may happen to be dealing.

From the local point of view, the chief aspect of the subject would appear to be the difference in thickness of the epithelial layer of the skin. In comparing man and mouse in this respect the difference is of course very great, the epithelial layer of the skin of a young healthy mouse being a delicate film but two cells in thickness, and, when rendered hyperplastic by applications of a carcinogenic agent, it will usually have attained the irreversible cancerous state before it has acquired a thickness equal to that of the normal epithelial layer of the skin of man. Thus the basal layer of the skin of a mouse will be relatively very accessible to the influence of a carcinogenic agent, and from a comparative point of view this will be especially the case with agents such as gas tars, they having on the whole more difficulty in reaching the basal layer than have mineral oils. Consequently we conclude that the difference in the carcinogenic potency of mineral oils as compared with tars for the skin of man is not so great as our mouse experiments would appear to suggest. It must not be inferred from these remarks that the susceptibility of an animal to a carcinogenic agent depends entirely upon the thickness of the epidermis, there is something much more subtle than this: compare the susceptibility of mouse, rabbit, rat and guinea-pig.

From the results of our experiments on the washing away of the mineral oil with soap and water it would, at first sight, appear to be very problematical whether any benefit to man would be forthcoming from the taking of a bath after contact with carcinogenic agents. In our experiments the removal of the surplus carcinogenic agents was apparently neutralised by the removal of the natural inhibitory substances represented by the fats of the skin. Further investigations, however, made it apparent that benefit should be derived from the washing. In the case of our first experiments the mineral oil was allowed six hours in which to act freely before any attempt was made to remove it by washing, and a lapse of six hours in the mouse represents a long interval in man. When the animals were washed with soap and water only one hour after the application of the carcinogenic mineral oil, there was an indication of definite protection to the animal, and as one hour for the mouse represents at least twenty-four hours for man, it is evident that if a bath is taken six to twelve hours after contamination with mineral oils, workers would do much to avoid subsequent skin troubles. The expectation of tumours in a batch of mice, washed with soap and water one-quarter to half an hour after contact with shale oil, should be very low indeed. As a matter of fact, we tested the effect of washing with soap and water six hours after the application of two weaker mineral oils, and in both instances there was here some delay in the tumour yield among the washed as compared with the unwashed animals.

We have performed many experiments in connection with the effect of alkalies besides those of washing with ordinary soap and water, but at present it is difficult to say how our results will fit in with future findings. For instance we found that a 1 per cent. watery solution of sodium hydrate applied a few hours previous to shale-oil applications delayed tumour formation somewhat more than did petroleum ether. Again, the carcinogenic potency of a synthetic tar, applied as a 1 per cent. solution in alcohol, was diminished in activity by the addition of 1 per cent. of caustic potash, but definitely increased when 1 per cent. sodium sulphide was substituted for the potash. The relative carcinogenic potencies were: tar in caustic potash, 66; tar in sodium sulphide, 181.

The effect of the agent on the general health of the animals is of prime importance. Poor general health means poor nourishment of the skin epithelium, and, as we know, a poorly nourished cell responds but feebly to the stimulation of the carcinogenic agent. The general health of man is not materially affected by the quantity of mineral oil or tar with which he may habitually come into contact during his particular occupation, but the health of the mouse may be profoundly affected. Among the most toxic agents we have utilised are unrefined lubricating fractions of mineral oils, and the low boiling "spirits"; the death-rates being here appreciably higher than among animals painted with gas tars. With enfeebled animals the yield of benign tumours may be lowered, at least towards the end of an experiment, but it is especially the change of the benign tumours to malignancy which is affected. This is partly the reason why there is relatively such a low malignant tumour yield among animals painted with shale oil as compared with gas tars giving a similar yield of benign tumours, and why a Pennsylvanian oil may induce as many malignant tumours among mice as a shale oil of similar viscosity, although the former is unquestionably of very much lower carcinogenicity for man. This is due to the animals remaining comparatively healthy under treatment with Pennsylvanian oil while their counterparts treated with shale oil became greatly debilitated, with consequently an inability to respond to the stimulation of the carcinogenic constituents present in the oil. It is essential to bear this point in mind when selecting oils for the lubrication of textile machinery, man obviously responding quantitatively differently to our animals, his general health being unaffected.

In Table XVIII we have given the results of a few selected experiments in order to illustrate the points under discussion. Careful scrutiny of the table reveals several items of interest. In the first place it will be noted that, with a given oil, there may be a larger yield of tumours from the acid-treated oil than from the untreated oil, provided the oil in question has a reasonable degree of carcinogenic activity and the quantity of acid utilised is not too great. We observe also that the increased yield is especially evident among the malignant tumours. While the unrefined product may be less actively carcinogenic than its refined counterpart, we have also noted that the addition of a saponifiable oil to the former may increase instead of decreasing its activity. The explanation of these phenomena would appear to lie in the direction of the relative amounts of inhibitory and truly carcinogenic constituents in the individual samples of oils with which we may happen to be

Table XVIII. *The effect of healthiness of tissues*

Oil no.	Stated origin	Remarks	Duration in weeks	Tumours B.	Tumours M.	Potency B.	Potency M.	Mean	R.P.
37	Shale	Unfinished lubricating	35	23	3	567	114	340	100
37	,,	6 % acid treated	35	39	6	175	51	113	34
39	,,	Finished lubricating	35	24	2	345	112	228	100
39	,,	6 % acid treated	35	35	10	96	50	73	32
46	Venezuelan	Unfinished spindle	40	29	6	304	50	177	100
47	,,	Finished spindle	40	40	11	167	70	119	67
55	Shale	Ordinary diet	35	29	3	378	110	244	100
55	,,	Enriched diet	35	48	18	474	176	325	133
8	,,	Ordinary diet	35	25	2	490	97	293	100
8	,,	Enriched diet	35	31	18	322	214	267	91
46	Venezuelan	Ordinary diet	40	29	6	304	50	177	100
46	,,	Enriched diet	40	55	7	483	71	277	156

B. Benign tumours. M. Malignant tumours.
Daily applications.

concerned. It seems that refining processes in general in the first place tend to remove the inhibitory substances preferentially, and that it is only when the refining becomes more drastic that the true carcinogenic compounds commence to be destroyed or removed. Further we herein find a partial explanation for the apparent difference in action of lanolin when applied for the treatment of mineral-oil and tar dermatitis and cancer. The inflammation-producing substances act both locally and generally in preventing tumour formation, and to a marked degree in preventing the change from benign to malignancy, by lowering the general vitality of the animal.

Similarly we find that an improved diet is conducive to a greater yield of tumours, with again predominance as regards the malignant variety; and in this case it is noticeable that there is not the tendency for delay in tumour arrival time, the carcinogenic potency being somewhat higher than when ordinary diet was used. In the acid-treated oil experiments, the advent of tumours was delayed, with consequently a lowering of the carcinogenic potency, but it is important to bear in mind that the delay was chiefly manifest as regards the benign tumours. A judiciously performed diet experiment, wherein the animals are painted bi-weekly only, will show another variant, the carcinogenic potency being markedly lowered with a startling delay in the advent of the malignant tumours.

DISCUSSION

In view of the discrepancy of some of the results obtained with tars and oils when lanolin was used as protective agent, it was natural that some explanation should be sought for. While we do not wish to speculate unduly, it may serve a useful purpose if we draw attention to a few of the possible reasons for the discrepancy as they occur to us.

1. Mineral oils in the pure state would appear to penetrate the skin tissues more easily than pure gas tars. It is to be presumed that lanolin penetrates more easily than either of the above carcinogenic agents, and consequently the admixture of lanolin with them, while it may assist penetration of the mineral oil, will not do so to anything like the extent that it does in the case of the gas tar. However, other fats and oils, such as olive oil, liquid paraffin, etc., will also assist the penetration of gas tars, and we have seen that the latter oils act in a direction opposite to that of lanolin, the yield of tumours being decreased instead of increased.

2. The addition of lanolin increases the viscosity of mineral lubricating oils and gas tars, and one would have expected a decreased yield of tumours in both instances, as was the case when a stiff petroleum grease was substituted for the lanolin. In view of the result with this petroleum grease, one can dismiss the possibility of the stickiness of the lanolin playing a major part in the increase in the yield of tumours with this substance when blended with pure gas tar. The mechanical effect of stickiness may, however, materially influence the blends of lanolin with the tar diluted with chloroform.

3. Tars tend to dry up and cake on the back of the animal. This contingency does not arise when painting with mineral oils, and it might be thought that the solvent action of the lanolin on the tar and the prevention of caking was responsible for the increased activity of the tar. That the prevention of caking has much to do with the process is, however, belied again by the stiff grease experiment, but a solvent action of the lanolin on the specific carcinogenic components may play an important part in the increase in yield of tumours.

4. In the case of separate treatment with lanolin, and possibly to a less extent with blends, the lanolin may act mechanically and physically by hindering actual contact of the mineral oil and tar with the cells of the organism. The resulting effect would be a tendency to damp the action of carcinogenic, inflammation-producing and other constituents of the carcinogenic agents, and as the inflammation-producing (cancer-inhibiting) constituents are relatively abundant in tars as compared with oils, the sum of the effect of the lanolin is in a positive direction.

5. Gas tars in the pure state compared with mineral oils contain a large

amount of what we have designated cell toxins (phenols, etc.). Cancer, we are aware, will not easily supervene on intoxicated cells although very liable to occur in the neighbourhood of a damaged epidermis resulting from a wound, etc. Cancer is characterised essentially by an abnormal capacity of the cells for division and an abnormal capacity for invasion of neighbouring tissues, but neither of these functions can be performed to a degree adequate for the development of what we call cancer unless the cells concerned are in a good state of health. There is little question that to the naked eye lanolin materially benefits the skin superficially, the epidermis remaining smooth, soft and pliable in place of becoming hard, scaly and cracked. It would be easy to attribute the increase in yield of tumours with a tar-lanolin blend to a better general health of the basal cells of the epidermis, but we have to remember that to some extent at least both creosote and shale oil damage the epidermis, and blends or separate treatment of each with lanolin lowers the yield of tumours. It is more than likely, however, that the crux of the question rests upon the actual amount of the different toxic and carcinogenic constituents in the particular agent under consideration, support for which contention is partly found in the experiments conducted with dilutions of gas tar in volatile solvents.

Whatever theories may be advanced in an effort to explain some of our apparently paradoxical results, the fundamental fact remains that any agent tested by us with a carcinogenic potency on daily application of under 100 gives a substantially lower potency figure when the animals are treated with lanolin. Our results are remarkably similar whether we utilise as carcinogenic agent mineral oils, gas tar or creosote. From the practical point of view we need not concern ourselves with the discordant results obtained with blends of lanolin with the above three agents, but to gain knowledge of the mechanism of the action of the lanolin, and possibly thereby of the mechanism of the formation of cancer itself, we are pursuing energetically further experiments in this field.

Recommendations

Workers exposed to contact with mineral oils and tars, and most of their products should, in order to avoid the risk of subsequent dermatitis and cancer of the skin, rub into all parts exposed to contact with the oils a small quantity of a mixture of equal parts of anhydrous lanolin and olive oil. The ointment should be used before commencing the day's work. When work is finished the soiled parts should be thoroughly washed with soap and water and carefully dried, a small quantity of the lanolin ointment being again used if circumstances allow of this being done. The employer, for his part, should make every endeavour to see that, so far as possible, mineral oils utilised in his work come within the specification recommended by us elsewhere as relatively safe. He has as his guide the essential fact that, other things being equal, the lower the refractivity of a mineral oil the lower its carcinogenicity.

SUMMARY

1. A mixture of anhydrous lanolin with about equal parts of olive oil was the most efficacious ointment tested for protecting our animals from mineral-oil dermatitis and cancer.

2. Some commercial products consisting of mixtures of the alcohols and esters contained in lanolin were, on the whole, less efficacious: possibly the apparent benefit was mostly due to the olive oil with which they were diluted.

3. Olive oil, glycerine, commercial soaps, etc., gave varying degrees of protection, the last being particularly useful under some circumstances.

4. In experiments with gas tars, lanolin does not appear to afford protection when relatively small quantities of it are mixed with the tar or when relatively large doses of tar are applied to the animal, before or after lanolin treatment.

5. Where the experimental conditions appear to conform more to those prevailing among most tar workers, lanolin has a definite protective action.

6. Separate application of the carcinogenic agent and the prophylactic agent as a rule results in a lower yield of tumours than applications of an admixture of the two. In this respect it is to be noted that, where the animals were treated with lanolin separately, the quantity applied was several times greater than that of the tar itself (similarly in mineral oil experiments). This, of course, was not the case in our experiments with the lanolin blends.

7. Relatively more lanolin is required to protect against gas-tar dermatitis and cancer than is required to protect against toxic oils or synthetic tars. This is probably due to the presence in gas tars of special inhibitory substances. Note the analogy in the action of small quantities of acid on mineral oils, the potency being raised, whereas larger quantities may lower it.

(*MS. received for publication* 24. I. 1935.—Ed.)