Carcinogenesis Studies with Benzoyl Peroxide (Panoxyl Gel 5%)

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Several groups of hairless mice were given UV radiation with and without pretreatment with 7,12-dimethylbenz(a)anthracene (DMBA), 5% benzoyl peroxide in a gel (Panoxyl), and gel alone, in various combinations, with appropriate control groups included, in order to see whether benzoyl peroxide, which is known to enhance chemical skin carcinogenesis after a single, small dose of DMBA, also enhances UV carcinogenesis. The mice were observed for skin tumors, and all skin lesions were histologically investigated. The percentage of tumor-bearing animals with time is called the tumor rate, the total number of tumors occurring is called the tumor yield. Continual treatment with 5% benzoyl peroxide in gel twice a week, with or without a short pretreatment period of UV radiation resulted in only 2 skin carcinomas, which is remarkable, but not significant. Both Panoxyl and gel alone enhanced tumorigenicity significantly in animals pretreated with a single dose of 51.2 μg DMBA. There was no difference between the enhancement caused by Panoxyl and the gel as regards the tumor rate, but when measured as final tumor yield, Panoxyl was slightly more tumor-enhancing than gel alone. However, both Panoxyl and gel protected significantly against UV tumorigenesis (all tumors). There was no difference between the protective effect of the 2 types of treatment. Neither Panoxyl nor gel alone influenced significantly UV skin carcinogenesis (malignant tumors). It is concluded that under these experimental conditions both Panoxyl and gel alone tend to protect against the tumorigenicity and do not enhance the carcinogenicity of UV radiation in hairless mice, whereas both gel and Panoxyl enhance chemical carcinogenesis. The carcinogenic mechanisms may be different for UV and chemical carcinogenesis, respectively. *J Invest Dermatol 86:442–448, 1986*

Benzoyl peroxide is a white crystalline powder, which is unstable in pure form. It belongs to the group of free radical generating compounds, which are widely used in the chemical industry and in pharmaceutical preparations. Benzoyl peroxide is used, e.g., as a bleaching agent in food industry and as a catalyst in the plastics industry [1–3]. When absorbed, the substance is readily metabolized by the liver and is excreted in the urine as hippuric acid. It has low long-term toxicity and no known carcinogenic effect when taken in moderate doses per os [4,5]. However, it is a moderate skin irritant, and has been used to treat acne [6,7] and to improve wound healing [8,9].

In 1981 Slaga et al [10] reported that benzoyl peroxide provoked both papillomas and carcinomas when repeatedly applied to the skin of SENCAR mice after a single application of 7,12-dimethylbenz(a)anthracene (DMBA). When applied on its own it produced no tumors, but its strong promoting potency has been confirmed [11].

In 1983 Epstein [12] reported that repeated topical applications did not cause tumors in mouse skin previously exposed to UV radiation.

By 1981 a number of lotions, gels, and ointments containing benzoyl peroxide were being widely used to treat acne, and the report of Slaga et al [10] caused concern among the drug control agencies. A commonly used preparation in Norway is Panoxyl, which contains 5 g benzoyl peroxide per 100 g. This preparation is produced on license from Stiefel Laboratories (U.K.) Ltd. by A/S Farmaceutisk Industri, Oslo (AFI), who advise the users to apply the Panoxyl gel in the evening and wash it off the next morning. The effect on acne is very good.

Because of concern about the possible carcinogenicity of benzoyl peroxide in connection with sunshine, Statens Legemiddelkontroll (The Norwegian Medicines Control Authority) decided that Panoxyl gel should be sold only on prescription, as a precaution until further guidance was available. The official warning states that benzoyl peroxide has "a cancer-promoting effect" and that there is therefore a risk of skin cancer in connection with UV radiation. Users are advised to avoid sunbathing and the use of sunlamps. The preparation should be used for the shortest possible time. A text on the package says only that sun exposure must be avoided during treatment.

It was also agreed that our institute should start a study investigating the skin carcinogenicity of benzoyl peroxide in hairless mice, mimicking as far as possible the human situation by using Panoxyl twice a week in combination with both DMBA and UV radiation, mainly in cocarcinogenesis programs with alternating use of Panoxyl and UV radiation.

MATERIALS AND METHODS

Animals Male and female mice of the *hr/hr* Oslo strain, obtained from Gamle Bomholt Gaard, Aarhus, Denmark, were used. Spontaneous skin tumors have not been observed in these animals. All the mice were housed in plastic cages in the same room, 8 to a cage, with constant temperature and a 12-h (7:30 AM/7:30 PM) light/darkness cycle. They were fed a standard diet and water ad libitum. The cages were cleaned at noon twice a week. Each experimental group consisted of 32 mice, 16 males and 16 females.

Panoxyl Gel 5% A/S Farmaceutisk Industri (AFI), Oslo, Norway, provided benzoyl peroxide 5% in a gel composed of 40 g

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Abbreviations:
DMBA: 7,12-dimethylbenz(a)anthracene

0022-202X/86/$03.50

ethanol, scent, and colloidal magnesium, aluminum silicate, hydroxypropyl-methylcellulose, macrogollaurylether, and citric acid sufficient to produce an appropriate gel in about 45.5 g distilled water, packaged in a tube. The company also gave us a sufficient amount of the pure gel that serves as a base for the Panoxyl, and their licensor Stiefel Laboratories (UK) Ltd. gave financial support without any restrictions on the research programs.

UV Radiation Five Philips sunlamps (Fig 1) of the type HP 3114, UV + IR 360 W, IR 300 W, were arranged about 1 m above a table on which the cages of the animals to be irradiated were placed. The relative spectral energy distribution of these lamps is shown in Fig 2. The cages were covered with a wire netting to prevent the animals from running away during irradiation.

The lamps were located about 60 cm from each other. When measured with a UV-meter (Waldman GMBH & Co Werk für Lichttechnik), the intensity of the UV rays was 1.75 mW/cm^2 to 1.60 mW/cm^2 at table level below each lamp, and 0.9 mW/cm^2, midway between 2 lamps. The placing of each cage was therefore systematically changed at each radiation session.

Radiation took place twice a week (Tuesdays and Fridays), starting with 3 min of exposure the first week, 4 min the second week, 5 min the third week, etc., up to 12 min. After the first irradiation of 12 min, some of the animals were severely sunburned and a few ulcerations occurred. We therefore stopped radiation for 2 weeks, and then continued with 8 min twice a week for another 2 months. Then there were again signs of too heavy UV exposure, and again we stopped all exposure for 2 weeks, and from then on the animals were given 5 min radiation twice a week until the end of the experiment. The average UV dose after 5 min of radiation is about 400 mJ per cm^2.

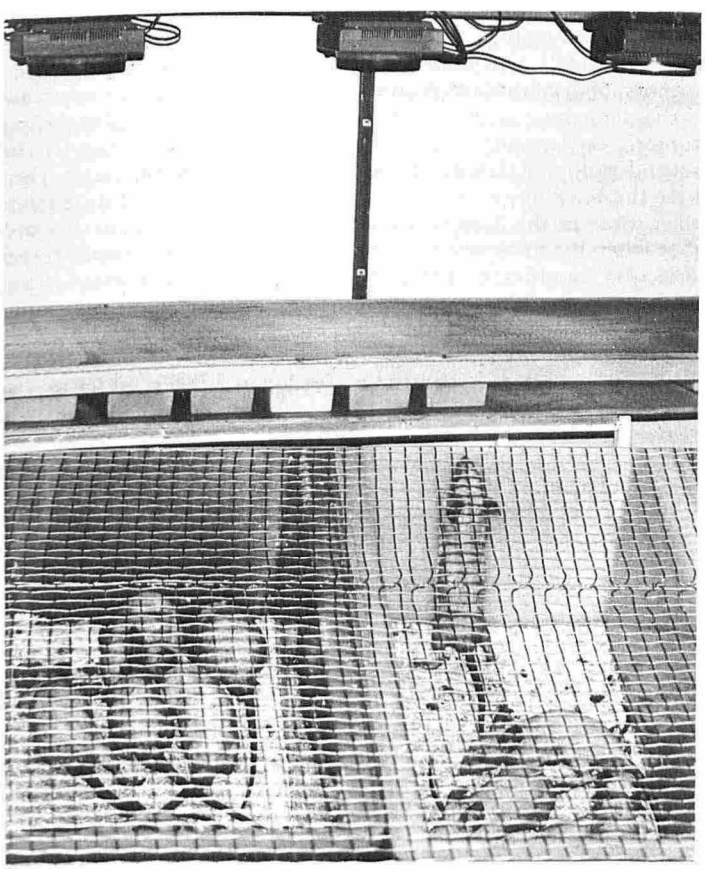

Figure 1. The experimental setup for UV irradiation of the mice. The upper panel shows the Philips sunlamps arranged over the table with the cages covered by the wire netting frame. The lower panel is a close-up of the cages covered with wire netting under which the hairless mice are visible.

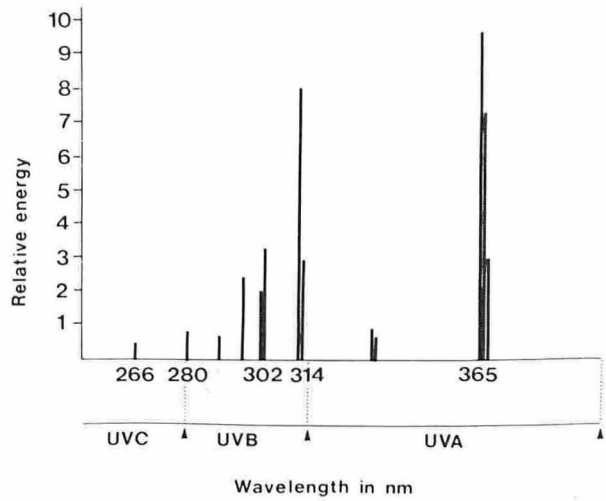

Figure 2. The relative spectral energy distribution of the Philips sunlamps HP3114. Most of the energy is in the UVB range, some of it in the UVA range, and very little in the UVC range.

The UV energy delivered by the lamps was repeatedly controlled. After about 6 months the lamps had faded a little. We then adjusted the radiation time, so that the dose given to the various groups remained approximately constant during the study.

Application of Panoxyl and Gel The technicians used surgical gloves, and an appropriate amount of Panoxyl or gel was rubbed gently with a finger into the skin of each mouse before each irradiation. The time between application of Panoxyl or gel and irradiation varied from 5 min to 30 min, but all the animals' skins were glistening when they were irradiated.

Experimental Groups Group 1 was given 51.2 μg DMBA in 100 μl acetone once, and thereafter left untreated for observation.

Group 2 was given 51.2 μg DMBA once and then gel twice a week throughout the experiment.

Group 3 was given 51.2 μg DMBA once and then Panoxyl twice a week throughout the experiment.

Group 4 was given 51.2 μg DMBA once and then treated with UV radiation twice a week.

Group 5 was given 51.2 μg DMBA once, and then gel was applied before UV radiation twice a week.

Group 6 was given 51.2 μg DMBA once, and then Panoxyl was applied before UV radiation twice a week.

Group 7 was given gel followed by UV radiation twice a week.

Group 8 was given Panoxyl followed by UV radiation twice a week.

Group 9 was treated twice a week with UV radiation.

Group 10 was given Panoxyl alone twice a week.

Group 11 was first given twice-weekly UV radiation for 3 weeks, 3 min radiation time the first week, 4 min the second week, and 5 min the third week. Thereafter the animals were treated with Panoxyl twice a week.

Observation of Tumors The crop of skin lesions was observed each week. A drawing was made of each animal, and each tumor was charted. The animals were observed for 60 or 61 weeks or until they were killed or found dead (see Table I). All animals were inspected weekly for skin lesions. When an animal had a large, ulcerating skin lesion which was obviously malignant, it was killed to prevent unnecessary suffering. An autopsy was performed on each animal, except when precluded by extensive autolysis. Histologic sections were made from all skin lesions and from the lungs of all animals, from the spleen when this was enlarged, and from other organs that were obviously diseased. All tumors registered as carcinomas have thus been histologically

Table I. Statistical Assessment of Tumor Rates and Tumor Yields for the Groups with Relatively Few Tumors Appearing[a]

	Tumor Rate				Tumor Yield		
	Overall (obs/exp)	1 vs 2 (obs/exp)	1 vs 3 (obs/exp)	2 vs 3 (obs/exp)	1 vs 2 (odds)	1 vs 3 (odds)	2 vs 3 (odds)
1. DMBA alone	0.76	0.86	0.82		1.00	1.00	
2. DMBA + gel	1.62	1.92		0.92	1.67		1.00
3. DMBA + Panoxyl	1.93		2.16	1.08		2.11	1.27
t	24.48	7.68	10.20	1.44	—[b]	—	—
One-tailed p value for positive trend	0.00001	0.0016	0.0001	0.3075	—	—	—
χ^2	17.41	8.68	14.35	0.25	5.44	11.26	0.80
Degrees of freedom	2	1	1	1	1	1	1
p value for heterogeneity	0.0002	0.0032	0.0002	0.6191	—	—	—
p value for tumor yield	—	—	—	—	$0.025 > p > 0.010$	$0.001 > p > 0.0005$	$0.40 > p > 0.30$
Conclusion	v.s.	s.	v.s.	n.s.	s.	v.s.	n.s.

Key: v.s. = very significant
s. = significant
n.s. = not significant
[a]For detailed explanation of statistics, see [12–14].
[b]— = no such parameter in statistical program

verified. Infiltration below the muscle was used as the main criterion of malignancy. In a few lesions, however, the cellular atypia and the infiltrative growth in the dermis were so striking that the lesion was classified as a carcinoma even when the tumor had not penetrated the muscle.

Statistical Evaluation The results are presented as tumor rates (the percentage of tumor-bearing animals in relation to the number of animals alive at the appearance of the first tumor related to time) and tumor yields (the cumulative occurrence of all skin tumors related to time) in all groups.

To evaluate differences in tumor rate, we have used the method for "non-incidental" tumors described by Peto [13] and elaborated with a computer-based test program by Peto et al [14]. This program takes into account varying mortality rates among the experimental groups, and assesses both the number of tumor-bearing animals and the time of the first tumor in each animal.

To evaluate the differences in tumor yield, we have used the method of Gail et al [15] based on "multiple times to tumor," Method 3. This method assesses the number of tumors appearing, varying mortality among the groups, and the time of appearance of each tumor.

Finally, the numbers of malignant tumors in each group were analyzed with the χ^2 test. Since this variable may not be normally distributed, the χ^2 analysis is only a rough estimate.

RESULTS

Survival In 4 groups (1, 2, 10, and 11) almost all the animals survived the whole observation period. In one group (3) 75% survived the whole observation period. In groups 4–9 inclusive, a large number of obviously malignant skin tumors developed. As mentioned, these animals were then killed and registered as carcinoma-bearing. Thus, in 4 groups (4, 5, 7, and 8) only 16–25% were left at the end of the experiment, and in 2 groups (6 and 9) only 3 and 6%, respectively, were left at 61 weeks. Hence, the survival curves cannot be used to analyze the influence of the treatment on the life length of the animals.

Tumors Appearing The results are presented in graphs illustrating tumor rates or tumor yields, and in tables for the statistical assessments. The results can conveniently be presented in 4 clusters as follows.

A. Treatment Groups (10 and 11) with no or Very Low Numbers of Tumors Appearing: The animals in group 11 developed no tumors. Those in group 10 developed no papillomas, but 2 squamous cell carcinomas of the skin and 1 lung adenoma were found. A χ^2 test of the 2 skin carcinomas in groups 10 and 11 compared with none at all in a group of 32 untreated animals gave a χ^2 value of 2.0313. With one degree of freedom, the p value is $0.20 > p > 0.10$, which is obviously not significant. Hence, mice treated with Panoxyl alone developed 2 carcinomas, but the ointment did not give rise to tumors in mice previously irradiated with UV rays for 3 weeks, and Panoxyl was thus not significantly tumorigenic by itself.

B. Treatment Groups (1, 2, and 3) with Fairly Low Tumor Incidence: The results are shown in Figs 3 and 4, the statistical analyses of all tumors in Table I, and of the final number of tumors in Tables II and III. Forty percent of the animals in group 1 developed tumors, 55% of those in group 2 got tumors appearing somewhat earlier than those in group 1, and 66% of those in group 3 acquired tumors, each tumor appearing slightly earlier than those in the second group. A similar trend was seen for tumor yields, but here the mice in group 3 developed tumors considerably earlier than those in the 2 other groups. Statistical assessment showed that when the groups were ranked as in Table I the overall trend was very significant. The difference between the DMBA + gel group and the DMBA + Panoxyl group was not significant; the other differences among the other groups were all significant or very significant. A χ^2 assessment of the final tumor yields in group 3 vs those in group 2, showed a χ^2 value of 5.0695, which gives a p value of $0.025 > p > 0.01$, which is significant (Table III). Hence, both Panoxyl and gel enhanced tumor development significantly in animals pretreated with 51.2 μg DMBA, and Panoxyl

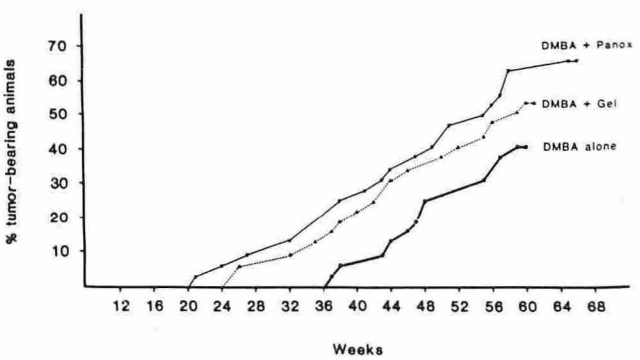

Figure 3. The tumor rate (i.e., tumor-bearing animals as percentage of those alive at appearance of the first tumor) during the observation period for the experimental groups 1, 2, and 3. Panox = Panoxyl, Gel = the base gel in which the benzoyl peroxide is delivered.

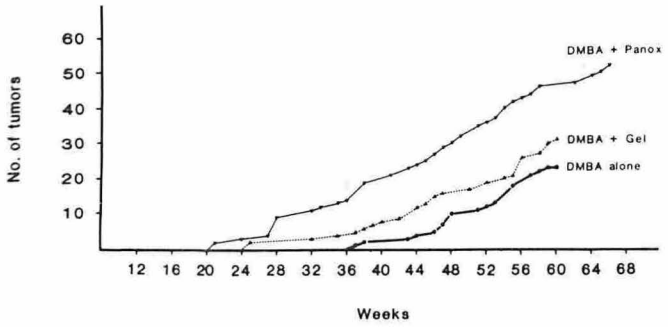

Figure 4. The tumor yield (i.e., total number of tumors occurring) during the observation period for the experimental groups 1, 2, and 3. Panox = Panoxyl, Gel = the base gel in which the benzoyl peroxide is delivered.

was only slightly more tumor-enhancing than gel measured as final tumor yield.

C. Treatment Groups (7, 8, and 9) with Moderately High Tumor Incidences: These are shown in Figs 5 and 6 as tumor rates and tumor yields, respectively, the statistical assessment of all tumors is shown in Table IV, and the final numbers of tumors in Tables II and III. As regards tumor rates, the animals in groups 8 and 9 developed about the same final tumor rate, 95%, but the mice in the group with UV radiation alone generally developed the tumors earlier than those in the UV + Panoxyl group. Those in group 7 had a slightly lower final tumor rate. The tumor yield curves showed a similar trend. The multigroup assessment of the results ranked according to Table IV showed a significant to a very significant trend. There was no significant difference between the use of gel combined with UV radiation and the use of Panoxyl combined with UV radiation. On the other hand, there were significantly more tumors after UV radiation alone than after UV combined with Panoxyl or gel. A χ^2 assessment of the final tumor yields showed no significant differences. Hence, in this experiment both the gel and the Panoxyl protected significantly against UV tumorigenesis (all skin tumors), and there was no difference between the 2 ointments.

Table II shows that UV radiation alone produced 44 malignant skin tumors, gel + UV 29, and Panoxyl + UV 51. A χ^2 test of the difference between Panoxyl + UV and gel + UV gave a value of 2.916, which gives a p value of $0.10 > p > 0.05$, which is not significant, but may be suggestive. A χ^2 test between the differences between gel + UV and UV alone, and Panoxyl + UV and UV alone showed no significant differences. Hence, as regards malignant tumors, neither gel nor Panoxyl influenced UV carcinogenesis (skin carcinomas) significantly, but the gel seemed to have a slight protective effect.

Table III. χ^2 Test Between Final Numbers of Tumors in Some Groups

	Group Comparisons		All Panoxyl vs All Gel
	3 vs 2	7 vs 8	
χ^2	5.0695	2.916	0.0428
Degree of freedom	1	1	1
p value	$0.025 > p > 0.01$	$0.10 > p > 0.05$	$0.90 > p > 0.80$
Conclusion	Significant	Suggestive	Not significant

D. Treatment Groups (4, 5, and 6) with a Very High Tumor Incidence: Fig 7 shows tumor rates and Fig 8 tumor yields. The statistical assessment of all tumors is shown in Table V, and of the final number of tumors in Tables II and III. All 3 treatments, group 4, group 5, and group 6, reached about the same final tumor rate, 97–100%. There was, however, a tendency for the DMBA + Panoxyl + UV treatment to give the earliest tumors, followed by DMBA + gel + UV and finally DMBA + UV alone. The same tendencies were seen for the tumor yields. However, here DMBA + UV alone resulted in 111 tumors, whereas in the 2 other groups the results were 135–140 tumors. A multigroup assessment with the groups ranked according to Table V showed a very significant trend. The only significant intergroup difference was between DMBA followed by UV radiation alone and DMBA followed by Panoxyl + UV. Hence, the tendency here was similar to the results seen in the moderately low tumor group (B), where initial painting with 51.2 μg DMBA was also involved. Table II shows that DMBA followed by UV radiation gave 27 malignant skin tumors, DMBA followed by gel + UV gave 32 malignant skin tumors, and DMBA followed by Panoxyl + UV gave 49 malignant skin tumors. There were no significant differences between these groups according to the χ^2 test.

E. Combined Results: Table VI shows the statistical assessment of some of the differences among the clusters of results marked B, C, and D. Each mode of treatment has been tested separately against all the others, but only the curves bordering on each other are expressed in the table. When the group with the highest tumor rates and yields in the cluster with relatively low tumorigenicity (group 3) was tested against the group with the lowest tumor rates and yields in the cluster with the medium carcinogenicity (group 7), the differences were very significant. Hence, continual UV radiation generally gave more tumors than pretreatment with 51.2 μg DMBA. When the group with the highest tumor rates and yields in the median carcinogenicity group (group 9) was tested against the group with the lowest tumor rate and yield in the high tumorigenicity group (group 4), the differences were

Table II. Final Numbers of Tumors Appearing in All the Groups

	No. Skin Tumors			Sum No. of Skin Tumors			No. of Other Tumors	
	Papillomas	Squamous Cell Carcinomas	Fibro-sarcomas	Malignant	Benign	Total No. of Tumors/No. of Animals	Lymphomas	Lung Adenomas
1. DMBA alone	18	4	0	4	18	22/32	2	3
2. DMBA + gel	31	0	0	0	31	31/32	1	2
3. DMBA + Panoxyl	49	3	0	3	49	51/30	0	3
4. DMBA + UV	84	20	7	27	84	111/28	1	0
5. DMBA + gel + UV	109	29	3	32	109	140/32	1	0
6. DMBA + Panoxyl + UV	86	47	2	49	86	135/30	1	6
7. Gel + UV	44	27	2	29	44	73/31	1	4
8. Panoxyl + UV	35	50	1	51	35	86/32	0	0
9. UV alone	49	43	1	44	49	93/30	0	1
10. Panoxylyl alone	0	2	0	2	0	2/32	0	1
11. UV init. + Panoxyl[a]	0	0	0	0	0	0/32	0	1

[a]Given 6 UV irradiations twice a week for 3 weeks before continual Panoxyl treatment twice a week.

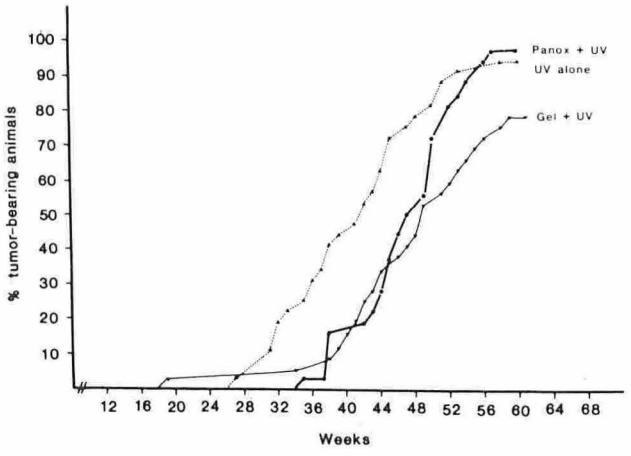

Figure 5. The tumor rate (i.e., tumor-bearing animals as percentage of those alive at appearance of the first tumor) during the observation period for the experimental groups 7, 8, and 9. Panox = Panoxyl, Gel = the base gel in which the benzoyl peroxide is delivered. UV = UV radiation from Philips sunlamps.

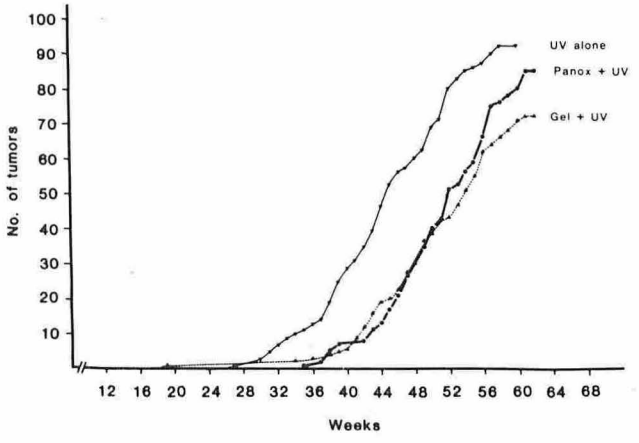

Figure 6. The tumor yield (i.e., total number of tumors occurring) during the observation period for the experimental groups 7, 8, and 9. Panox = Panoxyl, Gel = the base gel in which the benzoyl peroxide is delivered. UV = UV radiation from Philips sunlamps.

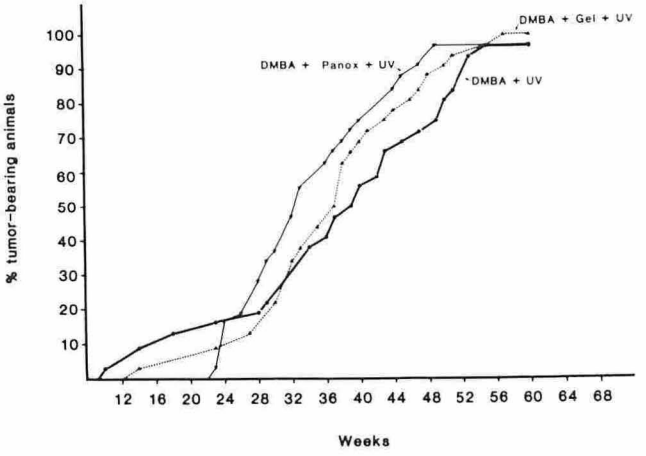

Figure 7. The tumor rate (i.e., tumor-bearing animals as percentage of those alive at appearance of the first tumor) during the observation period for the experimental groups 4, 5, and 6. Panox = Panoxyl, Gel = the base gel in which the benzoyl peroxide is delivered. UV = UV radiation from Philips sunlamps.

not significant. Hence, an additional, single dose of 51.2 μg DMBA initially did not significantly influence UV tumorigenesis.

Table VI also shows that when the rate and yield of tumors following UV radiation alone (group 9) were tested against the results of the highest tumorigenicity group (group 6) the differences were significant or very significant. Hence, Panoxyl enhances UV carcinogenicity when the latter is preceded by one application of 51.2 μg DMBA.

F. Conclusions: Under the experimental conditions described, both Panoxyl and gel enhance tumorigenesis when preceded by an application of 51.2 μg DMBA (with or without subsequent UV radiation), but both Panoxyl and gel protect against the tumorigenicity of UV radiation alone, and have no enhancement effects on UV carcinogenesis. In 64 mice treated with Panoxyl alone (32 of them first also irradiated for 3 weeks) 2 carcinomas occurred, which is statistically nonsignificant, but remarkable (see *Discussion*).

DISCUSSION

Our results have confirmed earlier studies [10,11,16] that benzoyl peroxide enhances chemical carcinogenesis induced by a single application of DMBA. We have also confirmed Epstein's findings [12] that benzoyl peroxide does not enhance UV tumorigenesis.

Table IV. Statistical Assessment of Tumor Rates and Tumor Yields for the Groups with a Medium Frequency of Tumors[a]

	Tumor Rate				Tumor Yield		
	Overall (obs/exp)	7 vs 8 (obs/exp)	7 vs 9 (obs/exp)	8 vs 9 (obs/exp)	7 vs 8 (odds)	7 vs 9 (odds)	8 vs 9 (odds)
7. Gel + UV	0.76	0.93	0.73		1.00	1.00	
8. Panoxyl + UV	0.88	1.07		0.74	1.05		1.00
9. UV alone	1.71		1.47	1.52		1.63	1.71
t	21.31	2.06	9.88	10.66	—[b]	—	—
One-tailed p value for positive trend	0.0006	0.2807	0.0019	0.0008	—	—	—
χ^2	13.33	0.34	8.38	9.96	0.06	6.75	8.01
Degrees of freedom	2	1	1	1	1	1	1
p value for heterogeneity	0.0013	0.5615	0.003	0.0016	—	—	—
p value for tumor yield	—	—	—	—	$0.90 > p > 0.80$	$0.010 > p > 0.005$	$0.005 > p > 0.001$
Conclusion	s. (v.s.)	n.s.	s.	s. (v.s.)	n.s.	s.	s.

Key: s. = significant
v.s. = very significant
n.s. = not significant
[a]For detailed explanation of statistics, see [12–14].
[b]— = no such parameter in statistical program

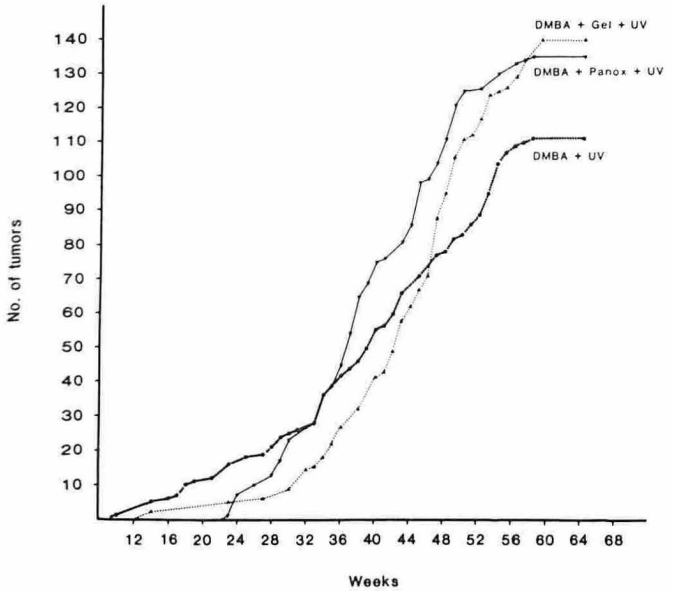

Figure 8. The tumor yield (i.e., total number of tumors occurring) during the observation period for the experimental groups 4, 5, and 6. Panox = Panoxyl, Gel = the base gel in which the benzoyl peroxide is delivered. UV = UV radiation from Philips sunlamps.

On the contrary, both Panoxyl and gel had a significant protective effect against UV tumorigenesis, and did not enhance UV carcinogenesis.

One weakness of this study is that the dose of UV radiation was relatively high. We had no prior experience of the sensitivity of these mice to UV radiation, and so we used a dose that first led to some ulcerations, and later on to tumors in about 90% of the animals. It might be objected that the tumor load after UV radiation alone was near the maximum of what the mice could sustain. However, when DMBA + gel + Panoxyl were given, the tumor yield increased, and hence UV radiation alone did not "saturate" the animals' capacity for producing tumors. In any case, it might have been better to have used a dose of UV radiation that would have led to tumors in about 50% of the animals; this might have given a clearer picture of the effects of Panoxyl, gel, and DMBA on putative increases or decreases in tumor rates and yields.

We applied the benzoyl peroxide in gel and the gel alone a few minutes before UV irradiation, and it is possible that the protective effect is due to a simple physical reflection of the rays through the layer of ointment. It is also possible that benzoyl peroxide and/or one of the chemical substances in the gel may have a specific filtering capacity for UV radiation. UV radiation may also split the benzoyl peroxide molecule. To this author's knowledge, this has never been investigated.

UV radiation by itself causes hyperplasia of the epidermis. Klein-Szanto and Slaga [17] have shown that benzoyl peroxide causes epidermal hyperplasia. Probably the rubbing in of gel alone also causes some hyperplasia. The enhanced hyperplasia caused by gel or Panoxyl may afford a slight protection from UV radiation. However, after initial treatment with a single dose of DMBA, the hyperplasia ought to be of the same or even increased degree [18], and in these cases both gel and Panoxyl enhanced UV carcinogenesis. Benzoyl peroxide and gel enhanced chemical skin carcinogenesis in mice also after a single starting dose of 51.2 μg DMBA, with or without UV radiation. Hence, it is not very probable that the induced epidermal hyperplasia is the cause of the protective effect.

A more unexpected finding was the enhancement of chemical carcinogenesis by the gel alone after a single application of DMBA. The gel consists of many chemical substances, of which hydroxypropyl-methyl-cellulose and macrogollaurylether may be the active ones. If an ointment base like the gel used here is an enhancer of chemical carcinogenesis, this would be a matter of some interest. It is too early to say whether or not the gel really has a very weak enhancement effect on chemical carcinogenesis. These indications will have to be confirmed in other studies. It is also very difficult to know whether a possible weak enhancement of this type in animal studies is relevant for assessment of a hazard to humans.

It is difficult to know whether 5% benzoyl peroxide in a gel used for the treatment of acne is hazardous for humans [19]. It may even protect against UV carcinogenesis when used in connection with sunbathing. The personal opinion of the author is that there should be little reason to worry, since the skin of young human adults is rarely in contact with strong chemical carcinogens. But the problem is not definitely solved.

The occurrence of 2 skin carcinomas in 32 mice treated with Panoxyl alone is remarkable. It is statistically not significant, and probably a random event, because 32 other mice that were first UV irradiated twice a week and then continually treated with Panoxyl for another 58 weeks developed no tumors at all. The occurrence of the tumors is remarkable, however, because spon-

Table V. Statistical Assessment of Tumor Rates and Tumor Yields for the Groups with Many Tumors Appearing[a]

	Tumor Rate				Tumor Yield		
	Overall (obs/exp)	4 vs 5 (obs/exp)	4 vs 6 (obs/exp)	5 vs 6 (obs/exp)	4 vs 5 (odds)	4 vs 6 (odds)	5 vs 6 (odds)
4. DMBA + UV	0.76	0.88	0.75		1.00	1.00	
5. DMBA + gel + UV	1.00	1.16		0.86	1.81	1.66	0.76
6. DMBA + Panoxyl + UV	1.45		1.47	1.19			1.00
t	19.86	4.32	10.18	5.12	—[b]	—	—
One-tailed p value for positive trend	0.0029	0.1216	0.0015	0.0828	—	—	—
χ^2	7.82	1.36	8.65	1.92	1.24	9.50	3.55
Degrees of freedom	2	1	1	1	1	1	1
p value for heterogeneity	0.0201	0.2431	0.0033	0.1656	—	—	—
p value for tumor yield	—	—	—	—	$0.20 > p > 0.10$	$0.005 > p > 0.001$	$0.10 > p > 0.05$
Conclusion	s. (v.s.)	n.s.	s.	n.s.	n.s.	s.	sugg.

Key: s = significant
v.s. = very significant
n.s. = not significant
sugg. = suggestive
[a]For detailed explanation of statistics, see [12–14].
[b]— = no such parameter in statistical program

Table VI. Statistical Assessment of Tumor Rates and Tumor Yields in Some Groups Bordering Each Other[a]

	Tumor Rate			Tumor Yield		
	3 vs 7 (obs/exp)	4 vs 9 (obs/exp)	9 vs 6 (obs/exp)	3 vs 7 (odds)	4 vs 9 (odds)	9 vs 6 (odds)
3. DMBA + Panoxyl	0.68			1.00		
7. Gel + UV	1.50			1.95		
4. DMBA + UV		0.92			0.89	
9. UV alone		1.10	0.74		1.00	1.00
6. DMBA + Panoxyl + UV			1.50			1.75
t	8.95	2.69	10.65		—	—
One-tailed p value for positive trend	0.0022	0.2276	0.002		—	—
χ^2	8.14	0.56	9.25	10.20	0.43	10.88
Degrees of freedom	1	1	1	1	1	1
p value for heterogeneity	0.0043	0.4552	0.0024	—	—	—
p value for tumor yield				$0.005 > p > 0.001$	$0.60 > p > 0.50$	$0.001 > p > 0.0005$
Conclusion	v.s.	n.s.	s.	v.s.	n.s.	v.s.

Key: v.s. = very significant
n.s. = not significant
s. = significant
[a]For detailed explanation of statistics, see [12–14].

taneous skin cancers in untreated hairless mice of this strain have never before been observed in our laboratory.

The authoritative opinion of a working group of the International Agency for Research on Cancer [1], published in February 1985, is clear, namely that there is only inadequate evidence for the carcinogenicity of benzoyl peroxide to humans, and consequently no evaluation could be made. They also stated that there is inadequate evidence for its carcinogenicity to experimental animals. The present results confirm this view.

It may objected that we applied gel and Panoxyl immediately before radiation, whereas humans are advised to use Panoxyl in the evening, and to wash it off the next morning. Obviously, it would have been interesting to have repeated the present study with a new protocol using this procedure and one in which Panoxyl and gel were applied to the skin some time after the UV radiation. We will start such experiments.

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