Tumors and Amyloidosis in Mice Painted With Crude Oil Found on Bathing Beaches

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ABSTRACT

Oil lumps collected on the beaches of Israel in 1970, 1971 and 1973 were extracted with pure acetone and the extracts were used to paint the skin of mice twice weekly for 12 months. The oil lumps originated from crude oil spilled from tankers. The less recently collected oils induced papillomata and lymphomata in some animals. They were also more active than the recent oil in the induction of generalized amyloidosis.

Mice painted for 12 months with acetone alone developed amyloidosis to a similar extent as those painted with the oldest oil. In previously reported experiments, however, acetone was much less active than the oil in producing amyloidosis after 5 months of painting. The possibility that acetone and oil might act both synergistically or to be antagonistic at different phases of amyloidogenesis is discussed.

INTRODUCTION

Oil spillage with a consequent contamination of bathing beaches is a world-wide problem. The presence of oil on beaches represents an esthetic nuisance and a source of irritation for the bathers, who have to clean themselves from a dirty and sticky substance. The question whether the pollution of beaches by oil also represents a health hazard has never been investigated, except for a preliminary study by us (BARR-NEA and WOLMAN, 1972). In that study were described short term experiments in which painting of the skin of mice with oil polluting beaches produced amyloidosis and a few tumors. Different batches of oil seemed to vary in their capacity to induce amyloidosis. The present paper deals with a comparison between the effects of various batches of beach oil and extends the previous observations.

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METHODS

Black and sticky lumps of oil were collected on beaches situated between Tel-Aviv and Ashdod. These beaches lie 30-40 kilometers north of the oil port of Ashkelon. On each occasion the oil collected seemed to have been deposited at different times. Care was taken to include soft semi-liquid lumps as well as hardened material. Three collections were made: the first in the spring of 1970, the second in the spring of 1971, and the third in the spring of 1973. The oils were cleaned of sand and put in pure acetone for several months. The saturated acetone supernatants were used for painting the skin of mice. In the preliminary experiments (BARRNEA and WOLMAN, 1972) the ultraviolet absorption of these extracts was spectrophotometrically determined.

Random-bred, male ICR mice, 2 months of age at the beginning of the experiment, were used. They were divided into 5 groups, each of 30 animals. The mice were painted twice weekly in the lumbo-sacral region without any other treatment of the skin. The mice were painted as follows: group 1 - acetone; group 2 - "1970" oil; group 3 - "1971" oil; group 4 - "1973" oil; and group 5 - no painting. At the beginning of the experiment the oils were kept in acetone for 3 years, 2 years and 1 month respectively. Painting was continued for 12 months and animals found dead in the course of the experiment were discarded.

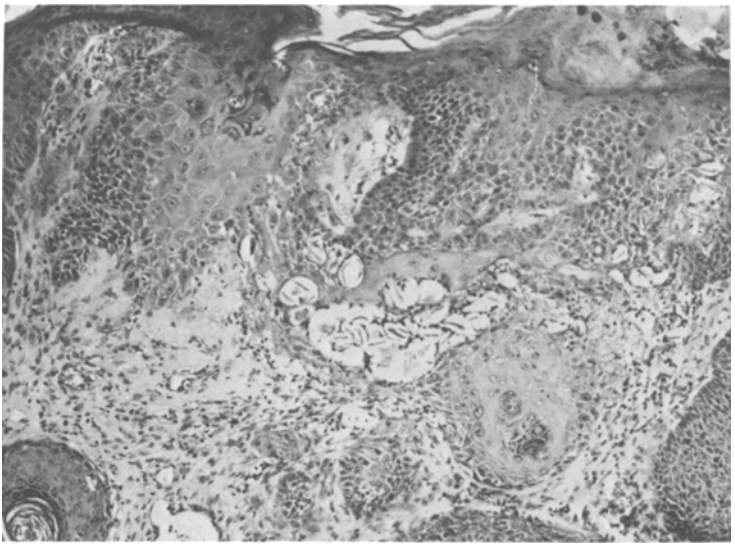
The mice were killed by cervical dislocation and the following tissues were fixed in formalin and processed: treated skin (and the same area in the non painted mice), non treated skin from the interscapular area, liver, heart, spleen, pancreas, kidney and adrenals. After paraffin embedding, sections were cut at 6-8 micrometers and were stained with H & E, Highman's Congo red (PEARSE, 1968) and standard toluidine blue (WOLMAN, 1971), and examined with bright field and polarized light microscopy. A positive Congo red polarization test was termed "presumptive amyloid", while a positive standard toluidine blue test (S.T.B.) was considered as "definitive amyloid".

RESULTS

Tumors.

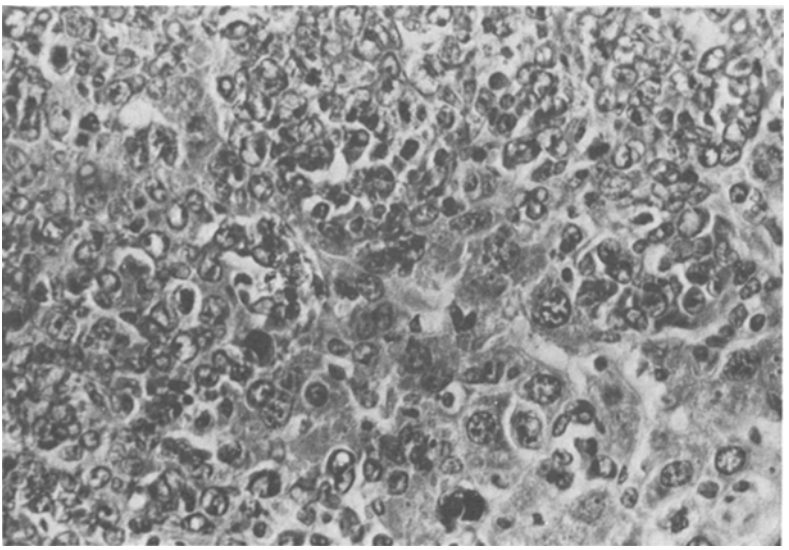
In 3 of 22 animals painted with the "1970" oil tumors were found in autopsy. One of the tumors was a skin papilloma possibly undergoing early malignant changes.

Figure 1. Papillomatous growth of skin painted with "1970" oil undergoing malignant change. H.E. x 175.



The other two were reticulum cell sarcomata which were found in one case in the liver and in the other in the liver and kidneys.

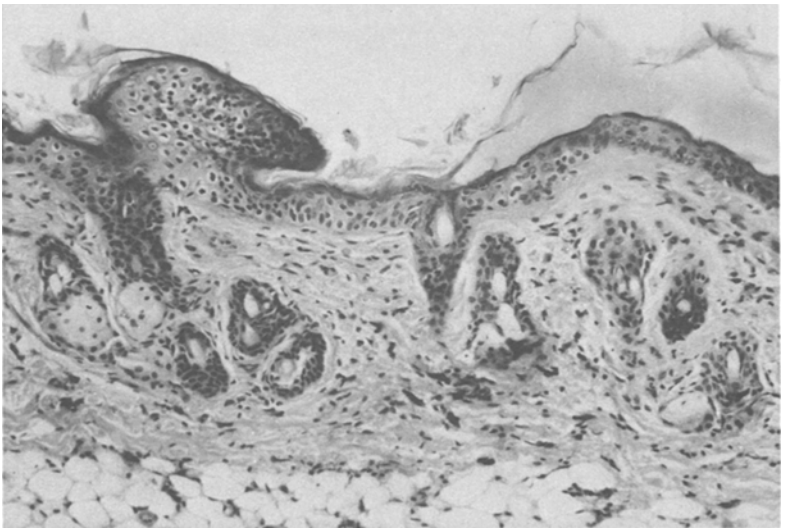
Figure 2. Reticulum cell sarcoma in liver of mouse painted with the "1970" oil. Cords of hepatic cells in upper left corner. Pleomorphic tumor cells occupy most of the field. H.E. x440.



One skin papilloma was found in the skin of a mouse painted with the "1971" oil. No tumors were found in any of the other animals (18 not painted, 23 acetone painted and 12 painted with the "1973" oil).

Papillomatosis and irregularity of the skin surface, sometimes associated with ulceration and scab formation, occurred in a high percentage of the painted skin. The impression was that oils with oncogenic activity produced more pronounced papillomatosis than the others, but this effect could not be quantitated as transition between folds and papillomatosis was gradual.

Figure 3. Papilloma on skin painted with the "1970" oil. H.E. x 175.



Amyloidosis.

Table 1 a & b indicates the incidence of amyloidosis as determined by the two procedures described above. The distribution of amyloidosis followed a fixed pattern in all groups. In minimal amyloidosis deposition occurred only in the painted and unpainted skin, where it often was seen as a delicate line at the epidermal basement membrane. With increasing severity amyloid masses were found in dermal papillae and sometimes extended along the adnexa and the blood vessels. The organs most often involved in amyloid deposition were in decreasing order of frequency: skin, heart, adrenal, kidney, liver and pancreas. In the heart amyloidosis involved only the cortex. In the kidneys the deposits occurred mostly in glomeruli. In the liver and pancreas amyloid deposits were found only in the wall of blood vessels. No amyloid was found in any of the 97 spleen sections which were available for examination. In group 1 mice, which were painted with acetone, amyloid deposition followed the same general pattern

as in the other groups with one significant difference: the involvement of blood vessels in the liver and the pancreas was rare (2/23) in comparison to group 2 (1970/oil) in which the incidence was 10/22.

TABLE 1

Incidence of amyloidosis and tumors in mice painted with acetone, acetone + oil, and untreated mice

a - Second experiment (12 months)

	Untreated Animals	Acetone	1970 oil	1971 oil	1973 oil
Presumptive Amyloidosis	2:18	12:23	10:22	11:22	2:12
Definitive Amyloidosis	1:18	12:23	10:22	8:22	0:12
Tumors	0	0	3	1	0

b - First experiment (5, 7 months) (BARR-NEA and WOLMAN, 1972)

	Untreated Animals	Acetone	1970 oil	1971 oil	Duration of Experiment
Presumptive Amyloidosis	0	1:9 8:24	6:10 10:17	1:9 -	5 months 7 "
Definitive Amyloidosis	0	0:9 2:24	3:10 4:17	0:9 -	5 months 7 "
Tumors	0	0	1	1	5 months

Non specific changes.

Painting with acetone and with acetone extracts was often associated with a number of changes. In many instances the painted skin had an irregular papillomatous appearance, often with loss of polarity of the epidermal cells and dyskeratosis, or else with atrophy and occasional ulceration. In a few cases infiltration by mast cells and eosinophils also occurred. Macroscopically there was loss of hair. In the liver, heart and adrenal occasional cells were undergoing hyaline change with marked cytoplasmic eosinophilia, loss of contact with neighboring cells and occasional single cell necrosis.

DISCUSSION

Tumors.

In the present experiments with 12 months of treatment, tumors occurred most frequently in mice painted with the "oldest" oil. By combining the present findings with those obtained in our preliminary experiments, the 1970 oil produced 4 tumors in 32 animals, the 1971 oil produced 2 tumors in 33 animals, while the 1973 oil, acetone treatment and normal aging (in the control animals) did not result in tumor formation. The tumor inducing capacity of oil might have increased with time, but it is also possible that the 1970 oil contained more carcinogenic substance than the later oil samples. The first possibility might correspond to the known fact that carcinogenicity of hydrocarbons is related to their degree of oxidation (BOYLAND and SIMS, 1965).

The occurrence of malignant lymphatic tumors in two animals might represent an incidental finding, but it is more likely that it is directly related to the painting with the 1970 oil.

Amyloidosis.

In the present study painting was continued for 12 months, compared to 2, 5, and 7 months in the preliminary communication (BARR-NEA and WOLMAN, 1972). The longer painting period is probably responsible for the wider distribution and changes in the characteristics of the deposited amyloid. While after 5 and 7 months of painting less than 50% of animals painted with the 1970 oil exhibited definitive amyloidosis, all the amyloids found in the present experiment were definitive (i.e. yielding a positive S.T.B. test). In the previous experiments painting for 5 months with the 1971 oil resulted in amyloid deposition in only 1/10 animals and the amyloid was presumptive in its characteristics. Aging of the oil, or the more prolonged treatment in the present experiment increased the rate of amyloidosis to 50%, most of which was definitive. Painting with the 1973 oil was associated with amyloidosis in only a few animals, in spite of the 12 months long treatment, and the amyloid was not definitive. These data indicate that aging of oils is probably the main factor in their pathogenic properties.

Amyloid deposition in both the present and previous experiments poses problems which are difficult to resolve. We previously found that after 7 months of painting with pure acetone 1/3 of the animals developed presumptive amyloidosis which was definitive in 2/8. In the present experiment about 50% of the animals painted for 12 months developed definitive amyloidosis. This, however, cannot mean that acetone was the main amyloidogenic agent, as mice painted for the same length of time with the most recent (1973) oil dissolved in the same batch of acetone had a negligible incidence of amyloidosis. The different results obtained by using the various oils indicate that some oils are either more active than others in amyloidogenesis, or exert less protective action against acetone-induced amyloidosis.

Table 1 b shows that the 1970 oil applied for short periods (5 months) induced amyloidosis (6/10 animals presumptive, 3 of each definitive), while acetone alone caused only minimal deposits (1/9 presumptive, none definitive). The time factor might, therefore, be of importance. It is possible that oil accelerates amyloid deposition, but after 12 months all the susceptible animals treated with acetone also developed amyloidosis. Although both factors are amyloidogenic, the presence of an inhibitory agent in fresh oils might complicate the picture, so that oils and acetone might be synergists or antagonists in different stages of oil aging and of amyloidogenesis. It might further be noted that no clinical data seem to be available to determine whether humans coming in frequent contact with acetone (for example, manicurists) are affected by dermal or other amyloidosis.

CONCLUSIONS

The present experiments indicate that repeated painting of mice with extracts of oils found on bathing beaches promote the development of tumors and amyloidosis. These observations cannot be easily extrapolated to human experience. Most persons exposed to the beach oil clean themselves and do not leave oil on their skin. It is not known whether cleaning with a solvent or a detergent protects the skin from the continued action of the oil, or helps some oil constituents penetrate the epidermis.

In mice the painting produced changes within about 5 months. Most bathers are exposed to oil for about ½ of each year. One month in the life of a mouse may be regarded as comparable to 3 years in the human. Assuming that humans react to the oil like mice, exposure to beach oil for about 30 years might be expected to increase the rates of tumor and amyloid incidence. Although this calculation is conjectural, it is believed that improved hygienic measures to avoid oil spillage might be preferable to a possible confirmation of the experiment in humans.

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